4AB

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 September 2004 (30.09.2004)

PCT

(10) International Publication Number
WO 2004/083818 A2

(51) International Patent Classification[7]: G01N

(21) International Application Number:
PCT/US2004/008327

(22) International Filing Date: 17 March 2004 (17.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/455,520 17 March 2003 (17.03.2003) US

(71) Applicant *(for all designated States except US)*: **RINAT NEUROSCIENCE CORP.** [US/US]; 3155 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **GRIMM, Jan** [DE/US]; 643 18th Avenue, Menlo Park, CA 94025 (US). **ROSENTHAL, Arnon** [US/US]; 150 Normandy Lane, Woodside, CA 94062 (US).

(74) Agents: **POLIZZI, Catherine, M.** et al.; Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, CA 94304 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/083818 A2

(54) Title: METHODS OF IDENTIFYING DRUG TARGETS AND MODULATORS OF NEURONS AND COMPOSITIONS COMPRISING THE SAME

(57) Abstract: The invention provides methods of identifying drug targets in dopaminergic and/or noradrenergic neurons and to the drug targets identified by such methods. This invention also provides a method of screening for agents that modulate dopaminergic neurons and/or noradrenergic neuron activity, function and/or drug target expression, and agents that bind drug targets and to kits for use in the methods described herein.

METHODS OF IDENTIFYING DRUG TARGETS AND MODULATORS OF NEURONS AND COMPOSITIONS COMPRISING THE SAME

CROSS RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. §119(e) to United States Patent Application No. 60/455,520 filed March 17, 2003, the contents of which are herein incorporated by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

FIELD OF THE INVENTION

[0003] This application is in the field of neuroscience, in particular, this invention relates to methods for identifying polynucleotide and polypeptide drug targets in dopaminergic and noradrenergic neurons, to drug targets identified by the methods described herein and to methods for screening for modulators of dopaminergic and noradrenergic neurons and compositions for use in the methods described herein.

BACKGROUND OF THE INVENTION

[0004] Dysfunction of midbrain dopaminergic and hindbrain noradrenergic neurons is central to the development of several neurological and psychiatric diseases or disorders. Midbrain dopaminergic neurons and their projections fall into three major systems (Airaksinen, M. S., et al (1997). *Eur J Neurosci* 9, 120-127). Nigrostriatal dopaminergic neurons reside largely within the substantia nigra pars compacta, project to the putamen and caudate nucleus. They comprise a key component of the voluntary motor system, and their degeneration leads to the development of Parkinson's disease, one of the most common neurodegenerative disorders with a prevalence of approximately 1 % in the population over the age of 65 with estimated costs exceeding $25 Billion each year in the United States alone (Abeliovich, A. et al (2000) *Neuron* 25:235-252).

[0005] Mesolimbic dopaminergic neurons reside in the ventral tegmental area and project to the amygdala, endorinal cortex and septum. These neurons influence emotional balance and addictive behavior (e.g. alcohol and cocaine). The abuse of recreational drugs is common in our society and has a major impact on our health care system with estimated costs exceeding $245.7 billion in 1992 in the US alone (www.nida.nih gov/Infofax/costs.html)) (Ambrozi, L. et al, (1976). *Br J Pharmacol* 58, 423P-424P). Mesocortical dopaminergic neurons reside in the ventral tegmental area and project to the neocortex in the frontal area. These neurons influence motivation, attention and planning. Hyperactivity of this pathway has been associated with schizophrenia. Approximately 1% of the population experience at least one schizophrenic episode at some time in their life with estimated annual costs - $32.5B in the (httl2://www.schizophrenia.com/newsletter/buckets/intro.html).

[0006] Incertohypothalamic dopamine neurons located in the most rostral portion of the medial zona incerta were originally described as the A13 tyrosine hydroxylase-containing group (Dahlstrom A, Fuxe K. (1965) *Experientia.* Jul 15;21(7):409-10.). Anatomical studies in rats suggest an involvement of the zona incerta in motor and oculomotor functions due to its connections with the pedunculopontine nucleus, the substantia nigra pars reticulata and the superior colliculus. Stereotactic surgery aimed at destroying the zona incerta area in Parkinsonian patients has been shown to relieve the motor symptoms, suggesting that structure might have a role in pathophysiology of the disease.

[0007] The largest collection of noradrenergic (NA) neurons in the central nervous system (CNS) is found in the locus coeruleus (LC). These neurons reside in the ventro-lateral region of the first hindbrain rhombomere and project to regions throughout the CNS. Their degeneration is associated with Parkinsons and Alzheimers disease (Chan-Palay, V. (1991). Alterations in the locus coeruleus in dementias of Alzheimers and Parkinsons disease. In Neurobiology of the Locus Coeruleus: *Progress in Brain Research*, C.D. Barnes and O. Pompeiano, eds. (Amsterdam: Elsevier Science Publishers)), whereas their abnormal function is thought to play a role in depression, sleep disorders (Siegel, M.J. (1999) *Cell* 98: 409–412.), and schizophrenia (Brier, B.et al., (1998). Norepinephrine and Schizophrenia: a new hypothesis for antipsychotic drug addiction. *In Catecholamines: Bridging Basic Science with Clinical Medicine*, Goldstein, D.S., Eisenhofer, G., and McCarty, R., eds. (Academic Press), pp 785–788.).

[0008] Patients with Parkinson's disease suffer from impaired motor function characterized by rhythmic tremor, inability to initiate and complete routine movement, muscle rigidity, postural instability and paucity of facial expression. The clinical symptoms are preceded by a selective loss of pigmented dopamine-producing neurons in the substantia nigra and ventral tegmental area in combination with a varying decay of the noradrenergic (locus coeruleus), cholinergic forebrain (nucleus basalis of Meynert) and serotoninergic (dorsal raphe nuclei) systems. The disease occurs sporadically in most cases, and the cause of cell death is not known, although viral infections, environmental toxins and oxidative stress induced by dopamine metabolites have been proposed. With loss of these neurons, excessive inhibitory stimuli are sent from the basal ganglia through the globus pallidus to the thalamus, leading to a decrease in the motor cortex activity and to the negative symptoms of Parkinson's disease; akinesia, bradykinesia and rigidity. In addition the loss of feed back loop between the nigral dopaminergic neurons and the thalamus leads to the release of spontaneous periodical impulse in the thalamus, which are responsible in part to the characteristic tremors (Deuschl, G.et al. (2000) *J Neurol* 247: Supp15, V33-48).

[0009] Not all midbrain dopaminergic neurons are equally susceptible to neurodegeneration in Parkinson's disease. Dopaminergic neuronal loss is most severe in the substantia nigra pars compacta while cells in the ventral tegmental area are less vulnerable (Hirsch et al., (1988) *Nature* 334:345-348). Within the substantia nigra pars compacta, the anatomical location and the expression of a variety of markers are associated with increased susceptibility to degeneration and loss. Neuronal loss tends to be greatest in the ventrolateral tier, followed by the ventromedial tier and dorsal tier (Farneley and Lees (1991) *Brain* 114 (Pt 5): 2283-2301.). This pattern of cell loss is specific to Parkinson's disease; it is the opposite of that seen in normal aging and differs from patterns found in striatonigral degeneration and progressive supranuclear palsy. It results in a regional loss of striatal dopamine, most prominently in the dorsal and intermediate subdivisions of the putamen, a process that is believed to account for akinesia and rigidity. This pattern of cell loss correlates with the expression level of dopamine transporter mRNA (Uhl et al., (1994) *Ann. Neurol* 35: 494-498). Neuromelanin-containing neurons are more susceptible to neurodegenertation while non-pigmented neurons are largely spared (Hirsch et al., 1988). Neuromelanin first appears in dopaminergic neurons within 3 years of birth and increases with age.

Neuromelanin is suspected to bind neurotoxins such as MPTP, paraquat or toxic metals or itself catalyze the production of toxic free radicals, providing a toxin pool within the pigmented neurons. It is, however, unlikely that neuromelanin is the sole causal factor for Parkinson's pathogenesis as it is accumulated in all humans with age. Differential expression of the calcium-binding proteins calbindin-D 28 kD and calretinin in a subset of midbrain dopaminergic neurons has been shown to be associated with neuroprotective advantage in Parkinson's disease (Tan et al., (2000) *Brain Res.*869:56-68). The calcium-binding proteins are found in the majority neurons in the ventral tegmental area, whereas in the substantia nigra less than 40% of the cells contained either calcium-binding protein. Gene inactivation studies in mice have shown that calbindin is not, however, causally involved in conferring resistance to neurotoxins and thus might only be used as a marker for less vulnerable cells (Airaksinen et al., (1997) *Eur. J. Neurosci.* 9:120-127).

[0010] In contrast to mesencephalic dopaminergic neurons, neurodegeneration of dopaminergic neurons in the hypothalamus is much less pronounced in Parkinson's disease. Different studies have revealed either none or only very limited loss of dopamine cells in several hypothalamic nuclei in Parkinson's brains (Purba et al.,(1994) *Neurology* Jan;44(1):84-9; Matzuk et al., 91985) *Ann Neurol* 5:552-5).

[0011] Quantitative analysis of degeneration of pigmented neurons in the locus coeruleus revealed that about 70% of the noradrenergic neurons are lost in Parkinson's disease. Cells in the rostral and caudal part are equally affected by the disease, in contrast to more pronounced loss of cells in the rostral part in the locus coerules that has been observed during normal ageing (Chan-Palay V, and Asan E. (1989) *Comp Neurol.* 287(3):373-92; Bertrand E. et al (1997) *Folia Neuropathol* 35(2):80-6).

[0012] The most accepted theory for the development of Parkinson's Disease (PD) involve the abnormal aggregation of a presynaptic protein designated alpha-synuclein, a 14 kd protein that was initially isolated from cholinergic nerve terminals of the Torpedo ray electric organ (Maroteaux et al. (1988) *J. Neurosci.*8: 2804-2815). Parkinson's Disease brain pathology is typified by the presence of abnormal protein aggregates, termed Lewy bodies, and selective loss of dopamine (DA) neurons. Alpha-synuclein appears to be the major protein component of these intra-cytoplasmic deposits in sporadic and familial forms of the disease (Mezey et al.(1998) *Nature Med.*4:755-756; Spillantini et al.(1998) *Proc. Natl. Acad. Sci* (USA) 95:6469-6473).

Direct evidence for the involvement of alpha-synuclein in Parkinson's Disease was provided by genetic studies of patients with rare, dominantly inherited variants of this disorder. Two independent pathological mutations have been described, a change from alanine to threonine at position 53 in Italian- American and Greek families (Polymeropoulos et al. (1997) *Science* 276:2045-2047), and a change from alanine to proline at position 30 in a family of German origin (Kruger et al. (1998) *Nat. Genet.* 18(2):106-8). These mutant proteins display a propensity to form Lewy body-like fibrils in vitro (Conway et al. (1998) *Nature Med.* 4: 1318-1320). Moreover, expression of the human alpha-synuclein mutation in transgenic mice results in Parkinson's Disease -like symptoms (Betarbet et al. (2002) *Bioessays* 24(2):308-318), while ablation of alpha-synuclein results in abnormal regulation of dopamine release (Abeliovich et al. (2000) *Neuron* 25:235-252). Unfortunately, despite the strong evidence for the involvement of alpha-synuclein in Parkinson's Disease its mechanism of its action and the genes involved in the process had not been yet identified.

[0013] Many different therapeutic approaches have been used in an attempt to counteract or compensate for the neural or chemical deficiencies that underline Parkinson's disease. The most effective treatment currently available is L-Dopa administration. L-Dopa is a precursor for dopamine, which crosses the blood brain barrier, and is taken up by the remaining dopaminergic neurons, converted to dopamine, which is secreted in the appropriate targets. L-Dopa compensates for the reduction in the level of the endogenous dopamine, increases the levels of dopamine in the striatum, and leads to a reversal or amelioration of the akinesia, bradykinesia and rigidity (Ambrozi et al. (1976) *Br. J. Pharmacol.* 58: 423P-424P). Unfortunately, it is not effective in reducing the tremors, nor does it slow the disease progression. Furthermore, after several years of treatment, L-Dopa leads to severe side effects and is no longer efficacious. Surgical lesions in the globus pallidus (pailidotomy) and electric stimulation of the subthalamic nuclei have been tried (both aimed at reducing the hyperactivity of the globus pallidus resulting from loss of dopaminergic neurons). However, although pallidotomy and electrical stimulation show promise in reducing akinesia and bradykinesia, especially akinesia that is induced by L-Dopa in advanced Parkinson's patients, they are not consistently effective in reducing the tremors. In addition, many symptoms recur after only a few years. A third therapeutic approach is grafting of dopamine-producing cells derived from fetal midbrain tissues, adrenal

medulla or carotid body. However, in a recent large clinical trial with human fetal neurons, no consistent therapeutic benefits were observed and some patients experienced severe side effects.

[0014] Schizophrenia is one of the most common mental illnesses, affecting about 1 % of the population, with an estimated cost to society of $32.5 billion per year in the US (U.S. Census Bureau and American Psychiatric Association). Schizophrenia is characterized by a constellation of distinctive symptoms that include thought disorder, delusions, and hallucinations. Thought disorder is the diminished ability to think clearly and logically. Often it is manifested by disconnected and nonsensical language. Delusions are common among individuals with schizophrenia, and are frequently paranoid or grandiose in nature. Hallucinations can be auditory, visual, olfactory or tactile. Most often they take the form of voices that may describe the person's actions, warn him of danger or tell him what to do. In addition, schizophrenics tend to be socially withdrawal, lack emotion and expression, and have reduced energy, motivation and activity. Sometimes schizophrenics exhibit catatonia where they become fixed in a single position for a long period of time. The first psychotic episode generally occurs in late adolescence or early adulthood, and often necessitates hospitalization where antipsychotic medication can commence under close supervision. Some persons with schizophrenia recover completely, and many others improve to the point where they can live independently, often with the maintenance of drug therapy. However, approximately 15 percent of people with schizophrenia respond only moderately to medication and require extensive support throughout their lives, while another 15 percent do not respond to existing treatment.

[0015] The proposal that schizophrenia is caused by an overactive dopamine system is based on the pharmacological findings that the drugs stimulating central dopamine receptors can produce a disorder indistinguishable from schizophrenia, and that anti-psychotic drugs block dopamine receptors (Davis et al. (1991) *Am. J. Psychiatry* 148: 1474-1486). However, whereas anti-psychotics block dopamine receptor activation soon after administration, therapeutic benefits are only seen after several weeks, suggesting that the primary defect in this disease may lie downstream of dopaminergic signaling. Thus, it is likely that other effectors have to be identified to address the cause of schizophrenia. The need for more effective anti-psychotic drugs not only stems from the limited effectiveness of such drugs in an appreciable number of schizophrenic patients but from the many side effects of such drugs.

Because these drugs block dopamine action, not surprisingly one of the most serious side effects of these drugs is the appearance of Parkinson's disease-like symptoms: tremor, muscle rigidity, loss of facial expression. Other side effects include dystonia, restlessness and tardive dyskinesia - involuntary, abnormal movements of the face, mouth, and/or body, which develop in about 25-40% of patients who take antipsychotic mediations for several years (http://www.schizophrenia.com/newsletter/buckets/intro htmi).

[0016] The role of noradrenergic neurotransmission in normal cognitive functions has been extensively investigated, however, the involvement in the cognitive impairment associated with schizophrenia has not been as intensively considered. The evidence of noradrenergic dysfunction occurring concomitantly with dopamine dysfunction in schizophrenia supports therapeutic approaches using noradrenergic drugs in combination with neuroleptics to enhance the treatment of cognitive impairment. Compared to typical antipsychotics (e.g. haloperidol), the newer atypical antipsychotics (e.g. risperidone and olanzapine) have greatly improved efficacy and exhibit less extrapyramidal motor side-effects. Acute treatment with atypical antipsychotics has been shown to induce c-Fos expression and transmitter release of locus coeruleus neurons (Ohashi, K et al. (2000) *Neuropsychopharmacology*, 23:162-9; Dawe, GS et al. (2001) *Biological Psychiatry*, 50:510-20).

[0017] Addiction is typically a chronic, relapsing brain disorder in which compulsive drug procurement and use dominate an individual's motivation (Tecott and Heberlein (1998) *Cell* 95:733-735). Drugs of abuse have been hypothesized to produce their rewarding effects by neuropharmacological actions on a common brain reward circuit of which the mesolimbic dopaminergic neurons are a key component. Natural rewards (e.g., sex and food) as well as addictive substances activate this reward circuit. Heroin, for example, increases the firing rate of dopaminergic neurons, whereas cocaine inhibits reuptake of dopamine. In addition to their acute effects, repeated use of psychomotor stimulants like cocaine and opiates like heroin produces changes in the mesolimbic dopamine system. Specifically, repeated use of cocaine or heroin can deplete dopamine from this system (Kish et al. (2001) *Neuropsychopharmacology* 24:561-567) These dopamine depletions may cause normal rewards to lose their motivational significance. At the same time, the mesolimbic dopamine system becomes even more sensitive to pharmacological

activation by psychomotor stimulants and by opiates (i.e., sensitization develops). These neuroadpative changes are probably critical for producing an addiction (De Vries et al. (1999) P*sychopharmacology(Berl) 143:254-260)*. Substances that activate the mesolimbic dopamine system without producing these neuroadaptive effects are probably not truly addictive.

[0018] Noradrenergic neurons in the LC express high levels of opioid receptors and plays a role in several effects of opioids, such as opioid dependence and withdrawal (Nestler EJ et al (1994) *Brain Res Bull* 35:521-528; Nestler EJ et al. (1997) *Science* 278:58-63). Systemic or intracoerulear administration of opioids, such as morphine, has been shown to have an inhibitory action on spontaneous LC neuronal activity (Korf J. et al. (1974*) Eur J Pharmacol.* 25:165-169). More recent studies suggest that administration of morphine does not simply decrease firing rates of LC neurons, but that it induces long-lasting synchronous oscillatory discharges in a subpopulation of LC neurons. These discharges may result in a facilitation of noradrenaline release in the widespread LC target areas (Zhu H and Zhou W.J (2001) *Neurosci* (21)21: RC179).

[0019] Dopaminergic and noradrenergic neurons have not been isolated free of other neurons and glial cells and only a few of the genes that are specifically expressed by these neurons have been identified. Identification of such genes (e.g., gene expression profiles) in, for example, in specific subsets of dopamine cells in Parkinson's disease that show different vulnerability will facilitate the identification key regulators that are involved in neuronal survival and potential drug targets for Parkinson's disease. Likewise, in the case of schizophrenia and drug addiction, the identification of genes that are specifically expressed in certain dopaminergic and noradrenergic neurons will provide novel candidates to target in the disease or addiction and a better understanding of the etiology of the disease or addiction. As dopaminergic and noradrenergic neurons are implicated in a variety of neurological diseases and disorders, there is substantial interest in identifying drug targets in these neurons and agents capable of modulating their activity. This invention provides such methods, drug targets and compositions for use in the methods.

[0020] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety. References include database sequences.

SUMMARY OF THE INVENTION

[0021] The invention relates, in general, to a method of identifying polynucleotide drug targets or polypeptide drug targets in dopaminergic and/or noradrenergic neurons and to the polynucleotide or polypeptide drug targets identified by such methods. This invention also provides a method of screening for agents that modulate neuron activity and/or function and/or gene expression via the polynucleotide or polypeptide drug targets and/or agents that bind to the polynucleotide or polypeptide drug targets identified by the methods described herein and to kits for use in the methods described herein.

[0022] In one aspect, the invention provides a method of identifying candidate drug targets in a dopaminergic and/or noradrenergic neuron comprising: (a) identifying and/or isolating a population of dopaminergic neurons (e.g., dopaminergic neurons in the substantia nigra pars compacta) and/or noradrenergic neurons; (b) evaluating the expression of one or more polynucleotides in the population of neurons, wherein the one or more polynucleotides and/or the one or more encoded polypeptides are candidate drug targets. The method may further comprise evaluating the expression of one or more polynucleotides in step (b) relative to a control population of neurons (e.g., whole brain).

[0023] In another aspect this invention relates to a method of identifying candidate drug targets in a population of dopaminergic or noradrenergic neurons comprising evaluating the expression of one or more polynucleotides in a dopaminergic or noradrenergic neuron population, wherein the one or more polynucleotides and their corresponding polypeptides are candidate drug targets.

[0024] In yet another embodiment this invention provides polynucleotide drug targets identified by the methods described herein and/or polypeptide drug targets identified by the methods described herein or combinations thereof and compositions and/or kits comprising the same.

[0025] In yet another aspect of the invention, microarrays comprising the polynucleotides and/or polypeptides of the invention are provided.

[0026] Yet another aspect of the invention relates to an antibody directed against the polypeptides of the invention. In some aspects the antibody modulates the activity and/or function of the polypetides.

[0027] In yet another aspect, this invention provides a method of assessing the ability of a candidate agent to modulate dopaminergic and/or noradrenergic neuron activity and/or function comprising: (a) contacting a population of dopaminergic and/or noradrenergic neurons expressing one or more drug targets (e.g., polynucleotide and/or polypeptide drug targets) with a candidate agent and (b) measuring the level of expression of the one or more drug targets in the population of dopaminergic and/or noradrenergic neurons, wherein an alteration of the level of expression of the one or more drug targets indicates the ability of the candidate agent to modulate dopaminergic and/or noradrenergic neuron activity and /or function and/or the therapeutic potential of the candidate agent for treating one or more diseases or disorders associated with dopaminergicand/or noradrenergic neuron activity or one or more symptoms associated with dopaminergicand/or noradrenergic neuron activity.

[0028] In one aspect, the method of assessing the ability of a candidate agent to modulate dopaminergic and/or noradrenergic neuron activity and/or function comprises measuring the level of expression of the gene transcripts for the one or more drug targets (e.g., RNA). In another aspect, the method of assessing the ability of a candidate agent to modulate dopaminergic and/or noradrenergic neuron activity and/or function comprises measuring the level of the polypeptide drug target.

[0029] In one embodiment this invention provides a method of screening for candidate agents that modulate dopaminergic and/or noradrenergic neuron activity, wherein the population of dopaminergic and/or noradrenergic neurons comprise, for example, nigrostriatal dopaminergic neurons in the substantia nigra pars compacta, mesolimbic and mesocotical dopaminergic neurons in the ventral tegmental area, hypothalamic dopaminergic neurons in the zona incerta (A13 group) and noradrenergic neurons in the locus coeruleus.

[0030] In another aspect this invention provides a method of assessing the ability of a candidate agent to modulate dopaminergic and/or noradrenergic neuron activity and/or function comprising: (a) contacting a population of dopaminergic and/or noradrenergic neurons expressing one or more drug targets (e.g., polynucleotide and/or polypeptide drug targets) with a candidate agent and (b) evaluating the activity and/or function of the population of dopaminergic and/or noradrenergic neurons, wherein an alteration in the dopaminergic and/or noradrenergic neuron activity indicates the therapeutic potential of the candidate agent

for treating one or more diseases or disorders associated with dopaminergic and/or noradrenergic neuron activity or one or more symptoms associated with dopaminergic and/or noradrenergic neuron activity.

[0031] In one embodiment this invention provides a method of screening for candidate agents that modulate dopaminergic and/or noradrenergic gene expression, wherein the population of dopaminergic and/or noradrenergic neurons comprise, for example, nigrostiatal dopaminergic neurons in the substantia nigra pars compacta, mesolimbic and mesocotical dopaminergic neurons in the ventral tegmental area, hypothalamic dopaminergic neurons in the zona incerta (A13 group) and noradrenergic neurons in the locus coeruleus.

[0032] In yet another aspect of this invention, a method of assessing the ability of a candidate agent to bind to one or more of the polynucleotide and/or polypeptide drug target identified by the methods described herein is provided.

[0033] Yet another aspect of this invention provides a method of staining nerve cells and maximizing isolation and/or recovery of polynucleotides (e.g., RNA) for use in the methods described herein.

[0034] Another aspect of this invention provides kits for use in the methods described herein.

BRIEF DESCRIPTION OF THE FIGURES

[0035] Figure 1. Impact of immunostaining procedure on RNA integrity. (a) Profile of RNA extracted from a cryosection from a fresh rat brain analyzed with the Agilent bioanalyzer. (b) RNA profile after immunostaining with our rapid staining protocol. The RNA is well preserved and shows a high 28S to 18S RNA ratio. (c) RNA content after immunostaining protocol with longer incubation with primary antibody (6 min) without addition of RNAse inhibitors to all buffers. The RNA is severely compromised and not suitable for amplification and microarray analysis.

[0036] Figures 2A-2B. Figure 2A shows identification of dopaminergic neurons in the substantia nigra pars compacta (SNc), the ventral tegmental area (VTA), the zona encerta (A13) and noradrenergic neurons in the locus coeruleus (LC). Rat brain sections were stained for tyrosine hydroxlase with the rapid staining protocol described herein. Figure 2B. Microdissection of immunostained tyrosine hydroxylase positive neurons from the substantia nigra pars compacta.

[0037] Figure 3. Integrity of RNA extracted from 3 different autopsy samples of the human substantia nigra. Sample a shows reasonable preservation of RNA while sample c contains degraded RNA that is not suitable for amplification and microarray analysis.

[0038] Figure 4. Microdissection of single pigmented neurons from the human subtanita nigra compacta.

[0039] Figures 5A-5B: Drug targets identified in zona encarta A13 neurons. Accession numbers of human orthologs are for TIGR Human Gene Index (THC numbers) and Genebank. The listed drug targets are expressed at least 8 fold higher relative to whole brain. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0040] Figure 6A-6B. Drug targets identified in locus coeruleus (LC) neurons. Accession numbers of human orthologs are for TIGR Human Gene Index (THC numbers) and Genebank. The listed drug targets are expressed at least 8 fold higher relative to whole brain. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0041] Figure 7A-7B. Drug targets identified in ventral tegmental area (VTA) neurons. Accession numbers of human orthologs are for TIGR Human Gene Index (THC numbers) and Genebank. The listed drug targets are expressed at least 8 fold higher relative to whole brain. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0042] Figure 8A-8B. Drug targets identified in substantia nigra (SN)neurons. Accession numbers of human orthologs are for TIGR Human Gene Index (THC numbers) and Genebank. The listed drug targets are expressed at least 8 fold higher relative to whole brain. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0043] Figure 9. Drug targets with higher expression in SN neurons relative to VTA neurons. Accession numbers of human orthologs are for TIGR Human Gene Index (THC numbers) and Genebank. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0044] Figure 10. Drug targets with higher expression in VTA neurons relative to SN neurons. Accession numbers of human orthologs are for TIGR Human Gene Index (THC numbers) and Genebank. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0045] Figure 11A-11C. Drug targets identified in human SN neurons. The listed drug targets are expressed at least 8 fold higher relative to whole brain. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0046] Figure 12. Drug targets identified in human LC neurons. The listed drug targets are expressed at least 8 fold higher relative to whole brain. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0047] Figure 13A-13C. Drug targets whose transcripts are expressed at least 4 fold higher in all catecholaminergic (CA) neurons relative to whole brain. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0048] Figure 14A-14T. Drug targets whose transcripts are differentially expressed in subsets of catecholaminergic (CA) neurons. Genes were filtered based on expression level relative to the whole brain reference (>4-fold higher or lower in 3/16 experiments) and transcripts with significant differences in expression between at least two cell groups were selected by multiclass SAM with a false discovery rate of <1%. The resulting set of genes and the experimental samples were grouped based on their similarities of gene expression by supervised hierarchical clustering (Pearson correlation, average linkage). Shaded areas indicate gene clusters. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0049] FIGURE 15A-15F. Drug targets whose transcripts are differentially expressed between SN and VTA neurons. Two-class significance analysis with a false disclovery rate cut-off of <1% was used to identify the genes. The sequences referenced in this Figure are herein incorporated by reference in their entirety.

[0050] Figure 16. In situ hybridization analysis with probes for tyrosine hydroxylase, hypothetical 38.5 kd protein and ZIP-4 demonstrates specific expression in the SN and VTA.

DETAILED DESCRIPTION OF THE INVENTION

[0051] Throughout the disclosure, various publications, patents and published patent specifications are referenced by an identifying citation. The disclosure of these publications, patents and published patent specifications are hereby incorporated by

reference into the present disclosure to more fully describe the state of the art to which this invention pertains.

Definitions

[0052] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology, microbiology, cell biology and recombinant DNA. [See, *e.g.*, Sambrook, et al. MOLECULAR CLONING: A LABORATORY MANUAL, 3rd edition (2001); SHORT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, et al. eds., 5th Edition (1995); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.); "PCR: A PRACTICAL APPROACH" (M. MacPherson, et al., IRL Press at Oxford University Press (1991); PCR 2: A PRACTICAL APPROACH (M.J. MacPherson, B.D. Hames and G.R. Taylor eds (1995)); ANTIBODIES, A LABORATORY MANUAL (Harlow and Lane, eds (1988)); and CULTURE OF ANIMAL CELLS (R.I. Freshney, ed. 4th Edition (2000)].

[0053] As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a gene" includes more than one or a plurality of genes, including mixtures or fragments thereof.

[0054] The term "polynucleotide" refers to polymeric forms of nucleotides of any length. The polynucleotides may contain deoxyribonucleotides, ribonucleotides, and/or their analogs. Nucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The term "polynucleotide" includes, for example, single-, double-stranded and triple helical molecules, a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, iRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A nucleic acid molecule may comprise, for example, naturally occurring nucleic acid molecules, synthetic or modified nucleic acid molecules.

[0055] The term "polypeptide" refers to polymeric forms of amino acids of any length and may include, but is not limited to, naturally occurring or modified amino acids.

[0056] The term "expression" includes production of a gene transcript and/or polypeptide.

[0057] The term "dopaminergic disease or disorder" refers to a disease or disorder in which the dopaminergic neurons are effected, involved and/or implicated. By way of example and not limitation such diseases or disorders include Parkinson's disease, schizophrenia or drug addition.

[0058] The term "noradrenergic disease or disorder" refers to a disease or disorder in which the noradrenergic neurons are effected, involved and/or implicated. By way of example and not limitation such diseases or disorders include Parkinson's disease, schizophrenia, drug addition or anxiety disorder.

[0059] As used herein, the term "modulate" refers to an alteration or modification in the function and/or activity of a dopaminergic and/or noradrenergic neuron. By way of example, such alteration or modification may include, but is not limited to, enhancement or diminishment of activity and/or function and/or and/or survival, enhancement and/or diminishment of symptoms associated with a dopaminergic and/or noradrenergic neuron activity and/or an amelerioation, mitigation of a disease or disorder and/or symptoms associated with a dopaminergic and/or noradrenergic neurons. Modulate is also intended to encompass enhancement or diminishment of polynucleotide drug target expression and/or polypeptide drug target expression in a dopaminergic and/or noradrenergic neurons

[0060] A "primer" is a short polynucleotide, generally with a free 3' -OH group that binds to a target or "template" potentially present in a sample of interest by hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target. A "polymerase chain reaction" ("PCR") is a reaction in which replicate copies are made of a target polynucleotide using a "pair of primers"or a "set of primers" consisting of an "upstream" and a "downstream" primer, and a catalyst of polymerization, such as DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are well known in the art, and taught, for example in "PCR: A PRACTICAL APPROACH" (M. MacPherson et al., IRL Press at Oxford University Press (1991)). All processes of producing replicate copies of a polynucleotide, such as PCR or gene cloning, are collectively referred to herein a "replication." A primer can also be used as a probe in hybridization reactions, such as Southern or Northern blot analyses. Sambrook et al., *supra*.

[0061] Reference to a Figure or Table herein is used to refer to any individual polynucleotide drug target listed in that Figure or Table or combinations of the

polynucleotide drug targets listed in the Table or Figure. When more than one Figure or Table is referenced herein, reference is to any individual polynucleotide drug target in the referenced Figures or Tables or combinations of the polynucleotide drug targets from any one or more of the Figures or Tables. Likewise, reference to a Figure or Table herein is used to refer to any individual polypeptide drug target encoded by any individual polynucleotide drug target listed in that Figure or Table or combinations of polypeptide drug targets encoded by the polynucleotide drug targets listed in the Table or Figure. When more than one Figure or Table is referenced herein, reference is to any individual polypeptide drug target encoded by any individual polynucleotide drug target in the referenced Figures or Tables or combinations of the polypeptide drug targets encoded by the polynucleotide drug targets from any one or more of the Figures or Tables.

[0062] The invention provides methods of identifying polynucleotide drug targets and/or polypeptide drug targets in dopaminergic and/or noradrenergic neurons, methods of screening for agents that modulate dopaminergic neurons and/or noradrenergic neuron activity, function and/or polynucleotide and/or polypeptide drug target expression. This invention also provides the polynucleotide and/or polypeptide drug targets identified by the methods described herein and to kits for use in the methods described herein. This invention is based on a discovery that anatomically and functionally distinct populations of dopaminergic neurons and/or noradrenergic neurons express distinct polynucleotides drug targets (e.g., gene expression profiles). As used herein, "drug target(s)" generally refers to polynucleotides and/or polypeptides identified by the methods described herein.

METHODS OF IDENTIFYING DRUG TARGETS

[0063] In one aspect this invention provides a method of identifying candidate drug targets in a dopaminergic and/or noradrenergic neuron comprising: (a) identifying and/or isolating a population of dopaminergic and/or noradrenergic neurons; (b) evaluating the expression of one or more polynucleotides in the population of dopaminergic and/or noradrenergic neurons, wherein the the one or more polynucleotides and/or the one or more encoded polypeptides are candidate drug targets. The method may further comprise evaluating the expression of one or more

polynucleotides in step (b) relative to a control population of neurons (e.g., whole brain, a population of neurons other than that being screened for drug targets).

[0064] In another aspect this invention relates to a method of identifying candidate drug targets in a population of dopaminergic or noradrenergic neurons comprising evaluating the expression of one or more polynucleotides in a dopaminergic or noradrenergic neuron population, wherein the one or more polynucleotides and their corresponding polypeptides are candidate drug targets.

[0065] Any population of dopaminergic and/or noradrenergic neurons may be used in the methods described herein. The population of dopaminergic and/or noradrenergic neurons may be obtained from a variety of sources and or samples. Examples include, but are not limited to mammals such as humans, primates or rodents (e.g., rats, mice), pathology, autopsy or biopsy samples, brain tissue banks, or in vitro cultures of dopaminergic and/or noradrenergic neurons. By way of example, but not limitation, midbrain dopaminergic neurons or noradrenergic neurons from the locus coeruleus can be used. The selection of the particular population of dopaminergic and/or noradrenergic neurons to be used in the method will, in part, be directed by the particular dopaminergic and/or noradrenergic neuron disease or disorder for which the drug target is being sought.

[0066] Generally, for evaluating the polynucleotide or gene expression profile of a population of dopaminergic neurons and/or noradrenergic neurons, the population of neurons must be identified and/or isolated from the other cells in the starting sample. The selected population of dopaminergic neurons and/or noradrenergic neurons can be identified by a variety of morphological and/or molecular criteria (e.g., anatomical location and/or known gene expression in conjunction with in situ or immunocytochemistry or pigmentation in human and primates). By way of example, dopaminergic and noradrenergic neurons can be identified by tyrosine hydroxolase immunostaining or, in primates or humans, by neuromelanin pigmentation. Subpopulations of dopaminergic neurons in the substania nigra that differ in their susceptibility to degeneration in Parkinson's disease can be selected by their anatomical location (e.g., ventral or dorsal part of the substania nigra) or expression of vunlnerability factors such as, for example, calbindin, capase-3 and/or glutamate receptors. Once identified the dopaminergic and/or noradrenergic neurons may be isolated by methods known in the art, including, but not limited to, laser microdissection (e.g., PALM Microlaser Technology).

[0067] Once the one or more neuronal cells of the dopaminergic and/or noradrenergic population are identified and/or isolated, the gene profile or gene expression cells can be evaluated by methods known in the art. Examples include, but are not limited to, PCR, microarray analysis in conjunction with RT-PCR, in situ or immunohistochemistry. By way of example, in situ hybridization in combination with a microarray can be utilized. Generally, the expression of one or more polynucleotides or gene expression profile of the dopaminergic and/or noradrenergic neurons is evaluated relative to the polynucleotide expression pattern of a control, such as, for example, whole brain tissue or a different population of neurons. Parameters for selecting candidate drug targets include, but are not limited to, polynucleotides and/or polypeptides specifically expressed in the dopaminergic and/or noradrenergic neurons relative to a control. Specifically expressed is intended to include, but is not limited to expression in the populations of the dopaminergic and/or noradrenergic neurons relative to absence of expression in the control or enhanced or diminished expression in the populations of the dopaminergic and/or noradrenergic neurons relative to the control. Statistical algorithms or commercially available statistical programs can be used to determine if the expression is statistically significant. By way of example, between about five to about eight fold and above difference in expression may be used to identify drug targets.

[0068] In a preferred embodiment, the method of the invention utilizes immunostaining and laser microdiscection for identification and/or isolation of the dopaminergic and/or noradrenergic neurons and in situ hybridization to evaluate the expression of the one or more polynucleotides. In this embodiment, the sample comprising the dopaminergic and/or noradrenergic neurons is sectioned and mounted on slides. Preferably, the sections are mounted on slides engineered for maximal laser cutting and catapulting efficiency. By way of example, a 1.35 μm polyethylene naphthalene membrane can be sealed to a slide with about 0.1% poly-L-lysine followed by UV irradiation for about 30 minutes. The slide with the membrane can be further treated with 0.1% poly-L-lysine for about 5 minutes and allowed to dry to further overcome the hydrophobic nature of the membrane and improve adherence of the tissue section the membrane/glass slide. The starting sample may be a dissected rat brain which or human brain sample, which was preferably immediately frozen on dry ice prior to use and/or stored at –80C until sectioning. Frozen tissue is sectioned

on the cryostat at, for example, about 12 micron thickness and on pre-processed polyethylene naphthalene membrane slides. Sections are fixed, preferably immediately in 100% ethanol for about 30 seconds followed by a brief dip in acetone (e.g., less than or about 2 -3 seconds) and air-dried at room temperature. RNA quality is greatly enhanced if the section are rehydrated in phosphate buffered saline (PBS) at a pH of about 7.0 to about 7.5 (higher pH leads to increased degradation of RNA) containing 1 about 1 to about 2 U/ul RNAse inhibitor (e.g., from Roche, Germany) for about 5 seconds. If the target population of neurons is dopaminergic or noradrenergic neurons, immunohistochemical staining to is utilized to detect tyrosine hydroxlase. Briefly, sections are stained with 100 µg/ml labeled primary antibody in PBS pH7 containing 1U/ul RNAse inhibitor (Roche, Germany) for 3 min. Tyrosine hydroxylase positive cells are detected with anti-tyrosine hydroxylase antibodies (e.g., clone TH-16, Sigma, USA). The antibody is purified with a protein A column and is covalently labeled with a fluorophore that has, for example, a succinimidlyl ester moiety that reacts with primary amines of proteins to form stable dye-protein conjugates. Kits are commercially for fluorophore labeling are commercially available, for example, the Alexa Fluor 488 monoclonal antibody labeling kit may be used following manufacturer's instructions. The labeled antibody is purified via gel filtration column chromatography followed by three washes in a buffered aqueous solution, such as PBS in a Microcon 30 centrifugal filter device. The sections are then washed in PBS (about, for example, pH 7.0) twice for 5 seconds, followed by dehydration for 30 seconds each in 75%, 95%, and 100% ethanol respectively and dried at room temperature.

[0069] Immunostained cells are dissected utilizing laser microdissection dissection (Schutze K and Lahr G. (1998) *Nat. Biotechnol* 16(8);737-742). By way of example, with a PALM Robot-Microbeam system (PALM microlaser technology, Germany) may be used. To facilitate detection of fluorescent cells, generally a drop of 100% ethanol is applied to the section while the cells are selected. Sections are allowed to air dry at room temperature for about 5 minutes and the cells, by way of example about 200 cells, are dissected and catapulted into about 30 µl of lysis buffer.

[0070] RNA may be isolated by conventional methodology. In a preferred embodiment total RNA is isolated by silica-gel spin columns after homogenization of the cells in a denaturing guanidine isothiocyanate containing buffer. By way of

example, a commercial kit such as the Picopure kit (Arcturus) may be used. In a preferred embodiment the RNA is amplified using T7-based linear amplification. By way of example, the RNA is amplified by two rounds of T7-based linear amplification (Van Gelder et al., (1990) *Proc. Natl. Acad. Sci* (USA) 87:1663-1667). Briefly, mRNA is converted into cDNA using an oligo-dT primer that contains a T7 RNA polymerase promoter site. The double-stranded cDNA is used as template for T7 RNA polymerase to transcribe antisense RNA which is amplified up to 1000 fold compared to the original input mRNA. The antisense RNA is used for a second round of amplification resulting in about 10^6-fold amplification. For amplification, by way of example, the Riboamp kit (Arcturus) was used according to the manufacturer's protocol. The reaction can be enhanced by the following modifications, to avoid generation of template-independent amplification product from the T7 primer, a five fold dilution of primer A was used for first round cDNA synthesis and the reaction volume was scaled down by 50%.

[0071] The amplification products can be characterized by a variety of methods known in the art. Nonlimiting examples include, assessment of the amplification product by microfluidic gel electrophoresis with, for example, with the Agilent bioanalyzer, hybridization. Products that show the expected amount and size distribution of RNA molecules (about 200 to about 2000 nucleotides) can be hybridized to DNA microarrays.

Polynucleotides

[0072] Another aspect of this invention is directed to isolated polynucleotide drug targets identified by the method described herein. The term polynucleotide is used broadly and refers to polymeric nucleotides of any length (e.g., oligonucleotides, genes, small inhibiting RNA, fragments of polynucleotides encoding a protein etc). By way of example, the polynucleotides of the invention may comprise the coding sequence for the active or functional domains of a protein or the intact protein and or non-coding sequences (e.g., regulatory sequences, introns etc). The polynucleotide of the invention may be, for example, linear, circular, supercoiled, single stranded, double stranded, branched, partially double stranded or single stranded. The nucleotides comprising the polynucleotide may be naturally occurring nucleotides or modified nucleotides. The polynucleotides referenced in Figures 5-15 and Tables 1-4, and/or their complements represent preferred embodiments of the invention. It is, however, understood by one skilled in the art that due to the degeneracy of the genetic

code variations in the polynucleotide sequences shown will still result in a polynucleotide sequence capable of encoding a drug target. Such polynucleotide sequences are therefore functionally equivalent to the sequence set forth in Figures 5-15 and Tables 1-4 and are intended to be encompassed within the present invention. Further, a person of skill in the art will understand that there are naturally occurring allelic variations of the polynucleotide sequences shown in Figures 5-15 and Tables 1-4 are also intended to be encompassed by the present invention

[0073] This invention also relates to homologs or orthologs of the polynucleotide sequences referenced in Figures 5-15 and Tables 1-4 and/or their complements. The homologs or orthologs may be identified by methods known in the art. A variety of sequence alignment software programs are available in the art to facilitate determination of homology or equivalence. Non-limiting examples of these programs are BLAST family programs including BLASTN, BLASTP, BLASTX, TBLASTN, and TBLASTX (BLAST is available from the worldwide web at ncbi.nlm.nih.gov/BLAST/), FastA, Compare, DotPlot, BestFit, GAP, FrameAlign, ClustalW, and PileUp. These programs are obtained commercially available in a comprehensive package of sequence analysis software such as GCG Inc.'s Wisconsin Package. Other similar analysis and alignment programs can be purchased from various providers such as DNA Star's MegAlign, or the alignment programs in GeneJockey. Alternatively, sequence analysis and alignment programs can be accessed through the world wide web at sites such as the CMS Molecular Biology Resource at sdsc.edu/ResTools/cmshp.html. Any sequence database that contains DNA or protein sequences corresponding to a gene or a segment thereof can be used for sequence analysis. Commonly employed databases include but are not limited to GenBank, EMBL, DDBJ, PDB, SWISS-PROT, EST, STS, GSS, and HTGS.

[0074] Parameters for determining the extent of homology set forth by one or more of the aforementioned alignment programs are well established in the art. They include but are not limited to *p* value, percent sequence identity and the percent sequence similarity. *P* value is the probability that the alignment is produced by chance. For a single alignment, the p value can be calculated according to Karlin et al. (1990) *Proc. Natl. Acad. Sci. (USA)* 87: 2246. For multiple alignments, the p value can be calculated using a heuristic approach such as the one programmed in BLAST. Percent sequence identify is defined by the ratio of the number of nucleotide or amino acid matches between the query sequence and the known sequence when the two are

optimally aligned. The percent sequence similarity is calculated in the same way as percent identity except one scores amino acids that are different but similar as positive when calculating the percent similarity. Thus, conservative changes that occur frequently without altering function, such as a change from one basic amino acid to another or a change from one hydrophobic amino acid to another are scored as if they were identical.

[0075] By way of example, polynucleotides of the invention are about 60%, more preferably greater than about 70%, even more preferably greater than about 80% and most preferably greater than 90% (e.g., 93% or 95 % or 98%) identity to one of the polynucleotide sequences referenced in Figures 5-15 and Tables 1-4 and/or their complements.

[0076] This invention also relates to a polynucleotide that hybridizes under stringent conditions to a polynucleotide referenced in Figures 5-15 and Tables 1-4. Hybridization reactions can be performed under conditions of different "stringency". Conditions that increase stringency of a hybridization reaction of widely known and published in the art. See, for example, Sambrook et al. (2001). Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25°C, 37°C, 50°C and 68°C; buffer concentrations of 10 X SSC, 6 X SSC, 4XSSC, 1 X SSC, 0.1 X SSC (where SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalents using other buffer systems; formamide concentrations of 0%, 25%, 50%, and 75%; incubation times from 5 minutes to 24 hours; 1, 2, or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6 X SSC, 1 X SSC, 0.1 X SSC, or deionized water. In a preferred embodiment hybridization and wash conditions are done at high stringency. By way of example hybridization may be performed at 50% formamide and 4X SSC followed by washes of 2x SSC /formamide at 50°C and with 1x SSC.

Polypeptides

[0077] Another aspect of this invention is directed to isolated polypeptide drug targets identified by the methods described herein. The term polypeptide is used broadly herein to include peptide or protein or fragments thereof. Examples of fragments include, but are not limited to, fragments comprising the active or functional domains of a protein. Also intended to be encompassed are peptidomimetics, which include chemically modified peptides, peptide-like molecules containing nonnaturally occurring amino acids, peptoids and the like, have the

selective binding of the targeting domains provided herein. ("Burger's Medicinal Chemistry and Drug Discovery" 5th ed., vols. 1 to 3 (ed. M. E. Wolff; Wiley Interscience 1995).

[0078] This invention further includes polypeptides or analogs thereof having substantially the same function as the polypeptides of this invention. Such polypeptides include, but are not limited to, a substitution, addition or deletion mutant of the polypeptide. This invention also encompasses proteins or peptides that are substantially homologous to the polypeptides.

[0079] A variety of sequence alignment software programs described herein above are available in the art to facilitate determination of homology or equivalence of any protein to a protein of the invention..

[0080] The term "analog" includes any polypeptide having an amino acid residue sequence substantially identical to at least one of the polypeptide sequences encoded by the polynucleotides referenced in Figures 5-15 and Tables 1-4 in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the functional aspects of the polypeptides as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid or another.

[0081] The phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue. "Chemical derivative" refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Examples of such derivatized molecules include for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are

those proteins or peptides which contain one or more naturally-occurring amino acid derivatives of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. Polypeptides of the present invention also include any polypeptide having one or more additions and/or deletions or residues relative to the sequence of a any one of the polypeptides whose sequences is described herein.

[0082] By way of example, polypeptides of the invention are at least about 60 %, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to any polypeptide encoded by a polynucleotide sequence referenced in Figures 5-15 and Tables 1-4. In some embodiments, the polypeptide is at least about 70% or 80% or 90% or 95% identical to any polypeptide encoded by a polynucleotide sequence referenced in Figures 5-15 and Tables 1-4.

[0083] Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

[0084] Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989, CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971, Comb. Theor. 11:105; Santou, N., Nes, M.,

1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

[0085] Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polypeptide sequence in the comparison window may comprise additions or deletions (i.e. gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical r amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Markers

[0086] One or more of the polynucleotide drug targets and/or the polypeptide drug targets identified by the methods described herein can be used as markers to identify a population of neurons. For example, substania nigra dopaminergic cells may be identified by expression of one or more of the polynucleotide drug targets referenced in Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 and/or their corresponding polypeptides, zona incerta A13 neurons may be identified by expression of one or more of the polynucleotide drug targets referenced in Figure 5, Figure 13, Figure 14 and Table 1 and/or their corresponding polypeptides, ventral tegmental area neurons may be identified by expression of one or more of the polynucleotide drug targets referenced in Figure 7, Figure 9, Figure 10, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 and/or their corresponding polypeptides, or neurons in the locus coeruleus may be identified by expression of the polynucleotide drug targets referenced in Figure 6, Figure 12, Figure 13, Figure 14, Table1 and Table 3 and/or their corresponding polypeptides.

METHODS OF SCREENING

[0087] This invention also provides for methods of screening for candidate agents that modulate the activity, function and/or expression profile of dopaminergic and/or noradrenergic neurons utilizing one or more polynucleotide drug targets and/or polypeptide drug target identified by the methods described herein. The screening assay may be performed either *in vitro* and/or *in vivo*. Candidate agents identified in the screening methods described herein may be useful as therapeutic agents for dopaminergic and /or noradrenergic diseases or disorders or one or more symptoms associated with dopaminergic and /or noradrenergic diseases or disorders. Examples of such diseases or disorders include, but are not limited to, Parkinson's disease, schizophrenia, drug addiction and anxiety disorders.

[0088] The one or more drug targets to be used in the screening method may be any polyucleotide drug target and/or polypeptides drug target identified by the methods described herein. The term polynucleotide is used broadly and refers to polymeric nucleotides of any length (e.g., oligonucleotides, genes, small inhibiting RNA, fragments of polynucleotides encoding a protein etc). By way of example, the polynucleotides of the invention may comprise the coding sequence for the active or functional domains of a protein or the intact protein and/or non-coding sequences. The polynucleotide of the invention may be, for example, linear, circular, supercoiled, single stranded, double stranded, branched, partially double stranded or single stranded. The nucleotides comprising the polynucleotide may be naturally occurring nucleotides or modified nucleotides. The polynucleotides referenced in Figures 5-15 and Tables 1-4 and/or their complement represent drug targets which may be used for screening. It is, however, understood by one skilled in the art that due to the degeneracy of the genetic code variations in polynucleotide sequences will still result in a polynucleotide sequence capable of encoding a drug target. Such polynucleotide sequences are therefore functionally equivalent to the sequence set forth in Figures 5-15 and Tables 1-4 and are intended to be encompassed within the present invention. Further, a person of skill in the art will understand that there are naturally occurring allelic variations of the polynucleotide sequences shown in Figures 5-15 and Tables 1-4 are also intended to be encompassed by the present invention. Additional examples of polynucleotides that may be used in the methods of screening for candidate agents include, but are not limited to, homologs or orthologs of the sequences referenced in Figures 5-15 and Tables 1-4 and polynucleotide that hybridizes under stringent conditions to a polynucleotide referenced in Figures 5-15 and Tables 1-4.

[0089] Likewise, one or more of the polypeptides identified as a drug target by the methods described herein may be utilized in the screening methods. The term polypeptide is used broadly herein to include peptide or protein or fragments thereof. Examples of fragments include, but are not limited to, fragments comprising the active or functional domains of a protein. By way of example, one or more of the polypeptides drug targets corresponding to the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4 may be used in the screening methods. Also intended to be encompassed are peptidomimetics of the polypeptides corresponding to the polynucleotides referenced in Figures 5-15 and Tables 1-4, polypeptides or analogs thereof having substantially the same function as the polypeptides corresponding to the polynucleotides referenced in Figures 5-15 and Tables 1-4 and polypeptides that are substantially homologous to the polypeptides corresponding to the polynucleotides referenced in Figures 5-15 and Tables 1-4.

[0090] The choice of the one or more drug targets will generally be directed by the population of neurons being screened. By way of example, for substania nigra dopaminergic cells one or more of the polynucleotide drug targets referenced in Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 and/or the polypeptides corresponding to the polynucleotides may be utilized, for zona incerta A13 neurons one or more of the polynucleotide drug targets referenced in Figure 5, Figure 13, Figure 14 and Table 1 and/or the polypeptides corresponding to the polynucleotides may be utilized, for ventral tegmental area neurons one or more of the polynucleotide drug targets referenced in Figure 7, Figure 9, Figure 10, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 and/or the polypeptides corresponding to the polynucleotides may be utilized, and for neurons in the locus coeruleus one or more the polynucleotide drug targets referenced in Figure 6, Figure 12, Figure 13, Figure 14, Table 1 and Table 3 and/or the polypeptides corresponding to the polynucleotides may be utilized.

[0091] In one embodiment the method of assessing the ability of a candidate agent to modulate dopaminergic and/or noradrenergic neuron activity and/or function comprises: (a) contacting a population of dopaminergic and/or noradrenergic neurons expressing one or more polynucleotide and/or polypeptide drug targets with a candidate agent and (b) measuring the level of expression of the one or more polynucleotide and/or polypeptide drug targets in the population of dopaminergic and/or noradrenergic neurons, wherein an alteration of the level of expression of the

one or more drug targets indicates the ability of a candidate agent to modulate dopaminergic and/or noradrenergic neuron activity and/or function and/or possible therapeutic potential of the candidate agent for treating one or more diseases or disorders associated with dopaminergic and/or noradrenergic neuron activity or one or more symptoms associated with dopaminergic and/or noradrenergic neuron activity. The method may further comprise evaluating the candidate agent in a control population of neurons (e.g., whole brain, a population of neurons other than that being screened).

[0092] Methods of evaluating polynucleotide and/or polypeptide expression are well known in the art and/or described herein. By way of example, polynucleotide microarrays maybe utilized. The candidate agent may alter expression of the drug target at any level including, but not limited to, modulating transcription of a polynucleotide drug target (e.g., a candidate agent that binds to the upstream controlling region) and/or modulating translation of the polynucleotide (e.g., an anti-sense polynucleotide, or a candidate agent which selectively degrades or stabilizes the mRNA, or by binding to drug target).

[0093] In another embodiment, a method of assessing the ability of a candidate agent to modulate dopaminergic and/or noradrenergic neuron activity and/or function comprises: (a) contacting a population of dopaminergic and/or noradrenergic neurons expressing one or more polynucleotide and/or polypeptide drug targets with a candidate agent and (b) evaluating the activity and/or function of the population of dopaminergic and/or noradrenergic neurons, wherein an alteration in the dopaminergic and/or noradrenergic neuron activity and/or function indicates the possible therapeutic potential of the candidate agent for treating one or more diseases or disorders associated with dopaminergic and/or noradrenergic neuron activity or one or more symptoms associated with dopaminergic and/or noradrenergic neuron activity. The method may further comprise evaluating the candidate agent in a control population of neurons (e.g., whole brain, a population of neurons other than that being screened).

[0094] Examples of parameters to measure to evaluate an alteration in dopaminergic and/or adrenergic function and/or activity when contacted with a candidate agent include, but are not limited to, gross phenotypic changes in the dopaminergic and/or adrenergic neurons, alteration in dopamine uptake in

dopaminergic neurons, neuronal excitability (Abeliovich et al. (2000) *Neuron* 25(1):239-52), neuronal survival, behaviorial changes or other deficits.

[0095] The candidate agent may be evaluated on dopaminergic and/or noradrenergic neurons *in vitro* or *in vivo*. *In vitro* systems include, but are not limited to cell cultures, such as primary cultures of dopaminergic and/or noradrenergic neurons. By way of example, primary cultures of doapminergic and/or noradrenergic neurons may be used (e.g., Hynes et al. (1994) *J. Neuroscience Res.* 37:144-154; Poulsen et al. (1994) *Neuron* 13:1245-1252; Masuko, S. et al (1986) *J. Neurosci.* 6(11):3229-41).

[0096] Alternatively, *in vivo* systems may be used in the screen. Any animal may be used for the screening method. Examples include, but are not limited to, drosophilia, zebrafish, rodents, such as mice or rats, or primates. The animal used in the screening method may naturally express one or more of the polynucleotide and/or polypeptide drug targets or transgenic animals expressing one or more of the polynucleotide and/or polypeptide drug targets may be generated by methods known in the art. Animal disease model systems may also be used. By way of example, mouse and rat models for Parkinson's disease include injection of 6-hydroxydopamine into the substantia nigra (rats, mice, cats or primates); intravenous infusion of Rotenone (rats), acute and chronic MTPT administration (mice, rats, primates) and mice or drosophila overexpressing alpha synuclein (Beal (2001) *Nat Rev Neurosci.* 2(5):325-34.).

[0097] Any population of doapminergic and/or neuroadrenergic neurons may be used in the screen. By way of example and not limitation, nigrostiatal dopaminergic neurons (e.g., substantia nigra), mesolimbic dopaminergic neurons (e.g., ventral tegmental area) and /or mesocotical dopaminergic neurons (e.g., ventral tegmental area) or noradrenergic neurons of the locus coeruleus may be screened by the methods described herein.

[0098] In yet another embodiment, a method of assessing the ability of a candidate agent to bind to one or more of the polynucleotide and/or polypeptide drug target identified by the methods described herein is provided. The method comprises, (a) contacting one or more of the polynucleotide and/or polypeptide drug targets for dopaminergic and/or noradrenergic neurons with a candidate agent and (b) evaluating the binding of the candidate agent to the polynucleotide and/or polypeptide drug target, wherein the ability of the candidate agent to bind to the drug target is indicative

of the possible therapeutic potential of the candidate agent for treating one or more diseases or disorders associated with dopaminergic and/or noradrenergic neuron activity or one or more symptoms associated with dopaminergic and/or noradrenergic neuron activity.

[0100] The drug targets to be used in assessing the ability of a candidate agent to bind to a drug target may be any one or more of the polyucleotide drug targets and/or one or more of the polypeptide drug target identified by the methods described herein. The choice of drug target will generally be directed by the population of neurons implicated in the dopaminergic and/or noradrenergic disease or disorder of interest. By way of example, for substania nigra dopaminergic cells one or more of the polynucleotides drug targets referenced in Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 and/or their corresponding polypeptide drug targets may be utilized, for zona incerta A13 neurons one or more of the polynucleotide drug targets referenced in Figure 5, Figure 13, Figure 14 and Table 1 and/or their corresponding polypeptide drug target may be utilized, for ventral tegmental area neurons one or more of the polynucleotide drug targets referenced in Figure 7, Figure 9, Figure 10, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 and/or their corresponding polypeptide drug target may be utilized, and for neurons in the locus coeruleus one or more of the polynucleotide drug targets referenced in Figure 6, Figure 12, Figure 13, Figure 14, Table 1 and Table 3 and/or their corresponding polypeptide drug targets may be utilized or combinations thereof.

[0101] By way of example, and not limitation, the ability of a candidate agent to bind to a drug target may be assessed by recombinantly expressing a polynucleotide encoding a drug target in a prokaryotic or eukaryotic expression system as a native or as a fusion protein in which a drug target polypeptide (or fragment thereof) is conjugated with a well-characterized epitope or protein as are well known in the art. Recombinant drug target polypeptide is then purified by, for instance, by immunoprecipitation using an antibody specific for the drug target or anti-epitope antibodies or by binding to immobilized ligand of the conjugate. An affinity column made of drug target polypeptide or drug target polypeptide fusion protein is then used to screen a mixture of candidate agents which have been appropriately labeled. Suitable labels include, but are not limited to flurochromes, radioisotopes, enzymes and chemiluminescent compounds. The unbound and bound

compounds can be separated by washes using various conditions (e.g. high salt, detergent) that are routinely employed by those skilled in the art. Non-specific binding to the affinity column can be minimized by pre-clearing the compound mixture using an affinity column containing merely the conjugate or the epitope. A similar method can be used for screening for agents that competes for binding to the drug target polypeptide. In addition to affinity chromatography, there are other techniques such as measuring the change of melting temperature or the fluorescence anisotropy of a protein which will change upon binding another molecule. For example, a BIAcore assay using a sensor chip (supplied by Pharmacia Biosensor, Stitt et al. (1995) *Cell* 80: 661-670) that is covalently coupled to native drug target or drug target fusion proteins, may be performed to determine the drug target polypeptide binding activity of different agents. Polypeptide microarrays comprising one or more of the polypeptide drug targets or fragments thereof attached to a support may also be used to screen for candidate agents capable of binding to the d to one or more polypeptide drug targets.

[0102] For an assay that determines whether a candidate agent inhibits transcription of a polynucleotide drug target, an in vitro transcription or transcription/translation system may be used. These systems are available commercially, and generally contain a coding sequence as a positive, preferably internal, control. A drug target polynucleotide is introduced and transcription is allowed to occur. Comparison of transcription products between an in vitro expression system that does not contain any agent (negative control) with an in vitro expression system that does contain a candidate agent indicates whether a candidate agent is affecting transcription of the drug target polynucleotide. Comparison of transcription products between the control and the drug target polynucleotide indicates whether the agent, if acting on this level, is selectively affecting transcription of the drug target polynucleotide (as opposed to affecting transcription in a general, non-selective or specific fashion).

[0103] For an assay that determines whether a candidate agent inhibits translation of a polynucleotide drug target, an in vitro transcription/translation assay as described above may be used, except the translation products are compared. Comparison of translation products between an in vitro expression system that does not contain any candidate agent (negative control) with an in vitro expression system that does contain a candidate agent indicates whether the agent is affecting

polynucleotide drug target transcription. Comparison of translation products between control and the drug target polynucleotide indicates whether the candidate agent, if acting on this level, is selectively affecting translation of the drug target polynucleotide(as opposed to affecting translation in a general, non-selective or specific fashion).

[0104] In another embodiment, competition assays are utilized. By way of example, an *in vitro* screening assay detects agents that compete with another substance (most likely a polypeptide) that binds a drug target polypeptide. Competitive binding assays are known in the art and need not be described in detail herein. Briefly, such an assay entails measuring the amount of a drug target polypeptide complex formed in the presence of increasing amounts of the putative competitor. For these assays, one of the reactants is labeled using, for example, ^{32}P.

[0105] By way of example, the ability of a candidate agent to modulate function or activity may be evaluated by, but are not limited to, gross phenotypic changes in the dopaminergic and/or adrenergic neurons, alteration in dopamine uptake in dopaminergic neurons, neuronal excitability (Abeliovich et al. (2000) *Neuron* 25(1):239-52), neuronal survival, behaviorial changes or other deficits.

[0106] It is also understood that the screening methods of this invention include structural, or rational, drug design, in which the amino acid sequence, three-dimensional atomic structure or other property (or properties) of a drug target 32polynucleotide or drug target polypeptide provides a basis for designing a candidate agent which is expected to bind to a drug target polynucleotide or polypeptide. Generally, the design and/or choice of agents in this context is governed by several parameters, such as the perceived function of the polynucleotide or polypeptide target, its three-dimensional structure (if known or surmised), and other aspects of rational drug design. Techniques of combinatorial chemistry can also be used to generate numerous permutations of candidate agents. For purposes of this invention, an agent designed and/or obtained by rational drug designed may also be tested in any of the methods described herein.

[0107] By way of example, the ability of a candidate agent to modulate function or activity may be evaluated by, but are not limited to, gross phenotypic changes in the dopaminergic and/or adrenergic neurons, alteration in dopamine uptake in dopaminergic neurons, neuronal excitability (Abeliovich et al. (2000) *Neuron* 25(1):239-52), neuronal survival, behaviorial changes or other deficits. Examples of

parameters to measure to evaluate an alteration in dopaminergic and/or adrenergic function and/or activity when contacted with a candidate agent include, but are not limited to, gross phenotypic changes in the dopaminergic and/or adrenergic neurons, alteration in dopamine uptake in dopaminergic neurons, neuronal excitability (Abeliovich et al. (2000) N*euron* 25(1):239-52), neuronal survival, behaviorial changes or other deficits.

[0108] The screening methods generally require comparison to a control sample to which no agent is added. The screening methods described above generally represent primary screens, designed to detect any agent that may the desired activity. The skilled artisan will recognize that secondary tests may be necessary in order to evaluate an agent further. For example, a cytotoxicity assay would be performed as a further corroboration that an agent which tested positive in a primary screen would be suitable for use in living organisms. Any assay for cytotoxicity would be suitable for this purpose, including, for example the MTT assay (Promega).

[0109] The drug targets identified herein may be used to generate transgenic animals or knockout animals by methods known in the art. By way of example, a knockout line(s) based on one or more drug targets identified herein will allow for assessment of phenotypic changes in the appearance number of dopaminergic and/or noradrenergic neurons (Cacalano et al.(1998) *Neuron* (21)1:53-62), behavior (Abeliovich et al.(2000) *Neuron* 25(1):239-252) of heterozygotes and homozygotes following birth and later stages of development. Standard histological methods can be used to compare homozygous and wild type animals at several stages throughout embryonic development (Moore et al. (1996) *Nature* 382(6586):76-79). Alternatively, the consequence of over expression or down regulation of the drug targets on dopamine release, dopamine reuptake and neuronal excitability can be evaluated in transgenic animals or *in vitro* cultures.

MICROARRAYS FOR SCREENING

[0110] The polynucleotide drug targets identified by the methods described herein are useful in the screening assays described herein. The screening method can be performed as described herein to detect polynucleotide sequences from the system in which the candidate agent was tested, which are complementary to the polynucleotide drug targets. By way of example, for the screening method the

polynucleotide sequences to be evaluated (e.g., polynucleotide drug targets) may comprise an array of one or more polynucleotide drug targets immobilized on a support (*e.g.*, dot blots on a nylon hybridization membrane Sambrook et al., or Ausubel et al) that is contacted with polynucleotides isolated from the system in which the candidate was evaluated. The one or more polynucleotide drug targets immobilized on the support may comprise all or part (e.g., a functional domain) of a coding region and/or non-coding sequences. One or more of the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4 and/or their complement represent drug targets which may be used for the microarray. By way of example, at least 2, 3, 5, 10, 20, 40, 50, 60, 70, 80, 90, 100, 200, 300 or 400 of the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4 and/or their complement may comprise the microarray. In some embodiments, 20, 30, 40 or 50 of the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4 and/or their complement may comprise the microarray. In some embodiments, 60, 70, 80, 90, 100, 200, 300 or 400 of the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4 and/or their complement may comprise the microarray.

[0111] The choice of the one or more polynucleotide drug targets comprising the microarray will generally be directed by the population of neurons implicated in the dopaminergic and/or noradrenergic disease or disorder of interest. By way of example, the microarray may comprise one or more of the polynucleotide drug targets referenced in Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 for screening for an agent that modulates drug target expression in substania nigra, one or more of the polynucleotide drug targets referenced in Figure 5, Figure 13, Figure 14 and Table 1 for screening for an agent that modulates drug target expression in zona incerta A13 neurons, one or more of the polynucleotide drug targets referenced in Figure 7, Figure 9, Figure 10, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 for screening for an agent that modulates drug target expression in ventral tegmental area neurons, one or more of the polynucleotide drug targets referenced in Figure 6, Figure 12, Figure 13, Figure 14, Table 1 and Table 3 for screening for an agent that modulates drug target expression in the locus coeruleus or combinations thereof.

[0112] In one embodiment, the microarray may comprise 2, 3, 5, 10, 20, 40, 50, 60, 70, 80, 90, 100, 200, 300 or 400 of the polynucleotide drug targets or their complements for substania nigra neurons, zona incerta A13 neurons, ventral

tegmental area neurons or locus coeruleus neurons or combinations thereof. In some embodiments, 20, 30, 40 or 50 of the polynucleotide drug targetsor their complements for substania nigra neurons, zona incerta A13 neurons, ventral tegmental area neurons or locus coeruleus neurons or combinations thereof may comprise the microarray. In some embodiments, 60, 70, 80, 90, 100, 200, 300 or 400 of the polynucleotide drug targetsor their complements for substania nigra neurons, zona incerta A13 neurons, ventral tegmental area neurons or locus coeruleus neurons or combinations thereof may comprise the microarray.

[0113] Microarrays may be a solid phase on the surface of which are immobilized a population of the polynucleotides of the invention. Microarrays can be generated in a number of ways. The one or more polynucleotide drug targets can be immobilized on solid support or surface, which may be made from, for example, glass, plastic (e.g., polypropylene, nylon), polyacrylamide, nitrocellulose, or other materials. Methods for attaching the nucleic acids to the surface of the solid phase include, but are not limited to, printing on glass plates (Schena et al. (1995) *Science* 270:467-470; DeRisi et al. (1996) *Nature Genetics* 14:457-460; Shalon et al.(1996) *Genome Res.* 6:639-645; and Schena et al.(1995) *Proc. Natl. Acad. Sci.* (U.S.A.) 93:10539-11286); or ink jet printer.

[0114] The microarrays can also be high-density oligonucleotide arrays. Techniques are known for producing arrays containing thousands of oligonucleotides complementary to defined sequences (see, Fodor et al. (1991) *Science* 251:767-773; Pease et al. (1994) *Proc. Natl. Acad. Sci.* U.S.A. 91:5022-5026; Lockhart et al. (1996) *Nature Biotechnology* 14:1675; U.S. Pat. Nos. 5,578,832; 5,556,752; and 5,510,270; Blanchard et al. *Biosensors & Bioelectronics* 11:687-690). Other methods for making microarrays may also be utilized (Maskos and Southern, (1992) *Nuc. Acids. Res.* 20:1679-1684; US Patent 6136592; WO 200054883; WO 200055363; WO 200053812; WO 200014273). The microarrays may be used as is or incorporated into a biochip, multiwell or other device. In general, the oligonucleotide probes range from about 6, 8, 10, 12, 15, 20, 30 to about 100 bases long, with from about 10 to about 80 bases being preferred, and from about 30 to about 50 bases being particularly preferred.

[0115] Preferably the microarrays of the present invention comprise, polynucleotides or fragments thereof from, for example, Figures 5-15 and Tables 1-4. One of skill in the art will understand that the hybridization and wash conditions are

chosen so that the nucleic acid sequences to be analyzed by the invention (e.g., the nucleic acids isolated from the test system) "specifically bind" or "specifically hybridize" to the nucleic acid sequences the array. Optimal hybridization conditions will depend on the length (e.g., oligomer versus polynucleotide greater than 200 bases) and type (e.g., RNA, or DNA) of probe and target nucleic acids. General parameters for specific (i.e., stringent) hybridization conditions for nucleic acids are described in Sambrook et al., (supra), and in Ausubel et al., 2001, "Current Protocols in Molecular Biology," Greene Publishing and Wiley-Interscience, New York).

[0116] Microarrays comprising one or more of the polypeptide drug targets or fragments thereof identified by the methods described herein are also useful in, for example, a screening assay to detect a candidate agent that binds to a polypeptide drug target. One or more of the polypeptide drug targets may be immobilized on a support that is contacted with a candidate agent. Methods for generating polypeptide microarrays and methods for evaluating binding of candidate agents to the polypetides comprising the microarray are know in the art (see, e.g., U.S. Patent Application Nos.: 2003/0049626, 2002/0106702, 2003/0013130, 2002/0110933; Koch et al (eds) Peptide Arrays on Membrane Supports: Synthesis and Applications (June 2002)Springer –Verlag).

[0117] The one or more polypeptide drug targets immobilized on the support may comprise an entire protein or portion thereof (e.g., functional or active domain). One or more of the polypeptide drug targets encoded by the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4 thereof may be used for the microarray. By way of example, at least 2, 3, 5, 10, 20, 40, 50, 60, 70, 80, 90, 100, 200, 300 or 400 of the polypeptide drug targets encoded by the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4 may comprise the microarray. In some embodiments, 20, 30, 40 or 50 of the polypeptide drug targets encoded by the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4 may comprise the microarray. In some embodiments, 60, 70, 80, 90, 100, 200, 300 or 400 of the polypetide drug targets encoded by polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4 may comprise the microarray.

[0118] As for the polynucleotide microarray, the choice of the one or more, polypeptide drug targets comprising the microarray will generally be directed by the population of neurons implicated in the dopaminergic and/or noradrenergic disease or disorder of interest. By way of example, the microarray may comprise one or more of

the polypetide drug targets encoded by a polynucleotide referenced in Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 for screening substania nigra neurons, one or more of the polypeptide drug targets encoded by a polynucleotide referenced in Figure 5, Figure 13, Figure 14 and Table 1 for screening zona incerta A13 neurons, one or more of the polypeptides drug targets encoded by a polynucleotide referenced in Figure 7, Figure 9, Figure 10, Figure 13, Figure 14 or Figure 15 and Tables 1, Table 2 or Table 4 for screening in ventral tegmental area neurons, one or more of the polypeptide drug targets encoded by a polynucleotide referenced in Figure 6, Figure 12, Figure 13, Figure 14, Table 1 and Table 3 for screening in locus coeruleus neurons. In one embodiment, the microarray may comprise 2, 3, 5, 10, 20, 40, 50, 60, 70, 80, 90, 100 or 200 of the polypeptide drug targets for substania nigra neurons, zona incerta A13 neurons, ventral tegmental area neurons or locus coeruleus neurons or combinations thereof. In some embodiments, 20, 30, 40 or 50 of the polypeptide drug targets for substania nigra neurons, zona incerta A13 neurons, ventral tegmental area neurons or locus coeruleus neurons or combinations thereof may comprise the microarray. In some embodiments, 60, 70, 80, 90, 100 or 200 of the polypetide drug targets for substania nigra neurons, zona incerta A13 neurons, ventral tegmental area neurons or locus coeruleus neurons or combinations thereof may comprise the microarray.

CANDIDATE AGENTS

[0119] Candidate agents suitable for assaying in the methods of the subject application may be any type of molecule from, for example, chemical, nutritional or biological sources. The agent may be a naturally occurring or synthetically produced. For example, the agent may encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 Daltons. Such molecules may comprise functional groups necessary for structural interaction with proteins or nucleic acids. By way of example, chemical agents may be novel, untested chemicals, agonists, antagonists, or modifications of known therapeutic agents.

[0120] The agents may also be found among biomolecules including, but not limited to, peptides, saccharides, fatty acids, antibodies, steroids, purines pryimidines, derivatives or structural analogs thereof or a molecule manufactured to mimic the

effect of a biological response modifier. Examples of agents from nutritional sources include, but is not limited to, extracts from plant or animal sources or extracts thereof.

[0121] Agents may be obtained from a may be obtained from a wide variety of sources including libraries of synthetic or natural compounds. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries or compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to random or directed chemical modifications, such as acylation, alkylation, esterification, amidification, *etc.* to produce structural analogs.

[0122] Exemplary types of candidate agents that may be screened in the methods include, but are not limited to, an antibody, an anti-sense molecule, a structural analog of a drug target, a dominant-negative mutation of a drug target, an immunoadhesion, and small molecules having a molecular weight of 100 to 20,000 daltons, 500 to 15,000 daltons, or 1000 to 10,000 daltons. Libraries of small molecules are commercially available.

[0123] By way of example, polynucleotides may be candidate agents. Examples of polynucleotides include but is not limited to, single-, double-stranded and triple helical molecules, a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, siRNA (small interfering RNAs), ribozymes, antisense, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. The polynucleotide may comprise naturally occurring nucleic acid molecules, synthetic or modified nucleic acid molecules.

Antibodies as Candidate Agents

[0124] The candidate agent may be an antibody which specifically binds one or more of the drug targets. The antibodies can be monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')2, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, heteroconjugate antibodies, single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including

glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies). The epitope(s) can be continuous or discontinuous. In one aspect, antibodies (e.g., human, humanized, mouse, chimeric) that can be made by using immunogens that express all or part of a polynucleotide encoding a drug target. In another aspect, an immunogen comprising a cell that overexpresses a drug target. Another example of an immunogen that can be used is all or part of a polypetide drug target. The antibodies may be made by any method known in the art and tested by known methods. In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. In another alternative, antibodies may be made recombinantly by phage display technology. *See*, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter *et al.*, *Annu. Rev. Immunol.* 12:433-455 (1994). Alternatively, the phage display technology (McCafferty *et al.*, *Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors.

KITS

[0125] The invention also provides kits for use in the instant methods. Kits of the invention include one or more containers comprising one or more polynucleotide and /or polypeptide drug targets provided by the method described herein, in the form of, for example, a microarray or antibodies. The kit may further comprise instructions for any of the screening method. The kit of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. In some embodiments, the kit comprises a container and a label or package insert on or associated with the container. The container holds a composition which is effective for use in the methods described herein. The container may further comprise an active agent. In another embodiment, the kit may comprise two or more containers each containing a composition effective for the methods described herein.

[0126] By way of example the kit may comprise one or more polynucleotide and/ or polypeptide microarrays as described above, wherein the one or more micoarray comprises gene expression profiles for substania nigra neurons, zona

incerta A13 neurons, ventral tegmental area neurons, and/or locus coeruleus neurons or combinations thereof. The gene expression profiles may be combined on a single microarray or two or more microarrays.

[0127] By way of example, the microarray may comprise one or more of the polynucleotide drug targets referenced in Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and 4 for screening for an agent that modulates drug target expression in substania nigra, one or more of the polynucleotide drug targets referenced in Figure 5, Figure 13, Figure 14 and Table 1 for screening for an agent that modulates drug target expression in zona incerta A13 neurons, one or more of the polynucleotide drug targets referenced in Figure 7, Figure 9, Figure 10, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 for screening for an agent that modulates drug target expression in ventral tegmental area neurons, one or more of the polynucleotide drug targets referenced in Figure 6, Figure 12, Figure 13, Figure 14, Table 1 and Table 3 for screening for an agent that modulates drug target expression in the locus coeruleus or combinations thereof.

[0128] By way of example, the microarray may comprise one or more of the polypetide drug targets encoded by a polynucleotide referenced in Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14, Figure 15, Table 1, Table 2 and Table 4 for screening substania nigra neurons, one or more of the polypeptide drug targets encoded by a polynucleotide referenced in Figure 5, Figure 13, Figure 14 and Table 1 for screening zona incerta A13 neurons, one or more of the polypeptides drug targets encoded by a polynucleotide referenced in Figure 7, Figure 9, Figure 10, Figure 13, Figure 14 or Figure 15, Table 1, Table 2 and Table 4 for screening in ventral tegmental area neurons, one or more of the polypeptide drug targets encoded by a polynucleotide referenced in Figure 6, Figure 12, Figure 13, Figure 14, Table 1 and Table 3 for screening in locus coeruleus neurons.

[0129] Alternatively the kit, for example, may comprise primers for amplifying the polynucleotide drug targets and/or antibodies which bind the polypeptide drug targets.

[0130] The following examples illustrate the manner in which the invention can be practiced. It is understood, however, that the examples are for the purpose of illustration and the invention is not to be regarded as limited to any of the specific materials or conditions therein.

EXAMPLES

Example 1: Drug Targets Identified In Rat Brain Tissue

Tissue preparation and immunohistochemisty

[0131] Standard histochemistry protocols result in severely degraded RNA that is not suitable for RNA amplification and microarray analysis. Incubation of tissue sections in buffered aqueous solutions results in nearly complete degradation after only several minutes. In order to allow microarray analysis of immunostained single cells a staining protocol that results in only minimal degradation of RNA was developed. The method involves a rapid 4-minute staining protocol that allows antigen detection with high sensitivity without severely compromising RNA integrity (Figures 1 and 2).

[0132] Rat brains are dissected and immediately and allow to freeze slowly on dry ice. Frozen brain specimen are stored at –80°C until sectioning. 12 µm serial sections are cut in the cryostat and are mounted on pre-processed polyethylene naphthalene membrane slides (see below). The sections are fixed immediately in 100% ethanol for 30 s followed by a dip in Acetone for 2 seconds and air dried at RT. The sections are rehydrated in PBS, pH7.0 containing 1U/ul RNAse inhibitor (Roche, Germany) for 5 seconds. The sections are stained with 100 µg/ml labeled anti tyrosine hydroxylase antibody (see below) in PBS pH7 containing 1U/ul RNAse inhibitor (Roche, Germany) for 3 min followed by two washes in PBS, pH7.0 for 5 seconds. The sections are then dehydrated for 30 s in 75%, 95%, and 100% ethanol respectively and air-dried at room temperature. (Fig 1).

Processing of slides for laser microdissection

[0133] Membrane slides were engineered for maximal laser cutting and catapulting efficiency: A 1.35 µm polyethylene naphthalene membrane is sealed to the slide with 0.1% poly-L-lysine followed by UV irradiation for 30 minutes. To overcome the hydrophobic nature of the membrane and improve adherence of the tissue section, the membrane coated glass slides are incubated again in 0.1% poly-L-lysine for about 5 minutes, spun dry and allowed to air dry for 1 hour.

Generation of Alexa Fluor 488 labeled antibody

[0134] Tyrosine hydroxylase positive cells are detected with anti-tyrosine hydroxylase ascites fluid (clone TH-16, Sigma, USA). The antibody is purified with a protein A column and is covalently labeled with Alexa 488 fluorophore using the

Alexa Fluor 488 monoclonal antibody labeling kit according to manufacturer's instructions. The labeled antibody is purified via gel filtration column chromatography followed by three washes with PBS in a Microcon 30 centrifugal filter device.

Laser Microdissection and RNA isolation

[0135] A PALM Robot-Microbeam system (PALM microlaser technology, Germany) for isolation of single neurons from frozen sections of brain tissues was used. The technology allows efficient contact-free isolation of cells of any size and shape while minimizing the risk of contamination. The selected cells are circumscribed with a high energy focused nitrogen laser resulting a gap of several microns in which any biological material has been ablated. The morphology of the adjacent tissue is not compromised by that procedure. Following laser-microdissection, the laser is focused slightly below the dissected target, which is then ejected from the object slide by photonic pressure of a second laser pulse and collected in a microcap containing RNA lysis buffer. To facilitate detection of fluorescent cells, a drop of 100% ethanol is applied to the section while the cells are selected. Sections are allowed to air dry again and 200 cells are dissected and catapulted into 30 µl of lysis buffer. Total RNA is isolated using silica matrix-based RNA isolation kit (Picopure Kit, Arcturus) contaminating genomic DNA is removed during the isolation by an on-column DNAse digestion step.

RNA amplification

[0136] RNA was amplified by two rounds of T7-based linear amplification (Van Gelder et al., 1990). In this procedure, the mRNA is converted into cDNA using an oligo-dT primer that contains a T7 RNA polymerase promoter site. The double-stranded cDNA is used as template for T7 RNA polymerase to transcribe antisense RNA which is amplified up to 1000 fold compared to the original input mRNA. The antisense RNA is used for a second round of amplification resulting in about 10^6-fold amplification. For amplification, the Riboamp kit (Arcturus) was used according to the manufacturer's protocol with the following modifications: To minimize generation of template-independent amplification product from the T7 primer, a five fold dilution of primer A was used for first round cDNA synthesis and the reaction volume was scaled down by 50%. The yield and size distribution of the amplified

aRNA product is evaluated by microfluidic gel electrophoresis with the Agilent bioanalyzer.

Preparation and hybridization of fluorescent labeled cDNA.

[0137] For each comparative array hybridization, labeled cDNA was synthesized by reverse transcription from amplified RNA from isolated neurons in the presence of Cy5-dUTP, and from the whole brain reference mRNA with Cy3-dUTP, using the Superscript II reverse-transcription kit (Gibco-BRL). For each reverse transcription reaction, 2 μg RNA was mixed with 3 μg random hexamers (Invitrogen) in 16 μl H2O, heated to 70 °C for 10 min and cooled on ice. To this sample, we added an 0.6 μl unlabelled nucleotide pool (20 mM each dATP, dCTP, dGTP; 4 mM dTTP and 16 mM aminoallyl-dUTP), 6 μl 5xfirst-strand buffer, 3 μl 0.1 M DTT and 2 μl of Superscript II reverse transcriptase (200 U/μl). The reaction was incubated five minutes at 25°C followed by one hour at 37°C and one hour at 42°C. The RNA was then degraded by adding 15 μl 1 N NaOH and incubating at 70 °C for 10 min and neutralized by addition of 15 μl 1 N HCl. The cDNA was purified by three rounds of centrifugation in a Centricon-30 micro-concentrator (Amicon). Each time 450 ul of H_20 was added and the reaction was concentrated to 20 μl. The purified samples were dried in a vacuum concentrator and reconstituted in 10 μl of 50mM Na_2CO_3 (pH 9). 1 μl of monofunctional NHS-ester Cy3 or Cy5 dye (Amersham, 10 mM in DMSO) was added to each sample and coupled in the dark for 1 h. Unreactive NHS-esters were quenched by addition of 4.5 μl 4 M hydroxylamine (Sigma) for 15 min in the dark. The labeled sample targets were combined with the respective reference targets and unincorporated Cy esters were removed by a silica based spin columns using the Qia-Quick PCR purification kit (Qiagen) according to manufacturer's protocol. The labeled targets were eluted in 2x 30 μl elution buffer. After addition Cot1 DNA (15 μg, Gibco-BRL), yeast t-RNA (15 μg, Sigma), ployA (15 μg, Sigma) and 420 μl H_2O, the labeled targets were concentrated to 10 μl in a Centricon-30 micro-concentrator (Amicon) and 2.5 μl deposition control targets (Operon) and 12.5 μl Deposition Hybridization buffer (Agilent) were added. The targets were denatured by heating for 2 min at 98 °C, centrifuged at 13,000 g for 5 min and placed on the array under a 22x 22 mm glass cover slip. Microarrays were hybridized for 48 h at 65 °C in a custom slide chamber with humidity maintained by a small reservoir of H20. Arrays were washed by submersion and agitation for 5 min in 0.5x SSC, 0.01% SDS, followed by

3 washes in 0.06x SSC for 3 min 3each. The arrays were dried by centrifugation for 2 min and scanned in a microarray scanner (Agilent). Images were analyzed with Agilent's feature extraction software. Data was filtered with respect signal significance (A two tailed t-test was used to determine significance of the signal versus background). Spot with a p-value of >0.01 were omitted. Only genes for which information was available for more than 80% of arrays were included. Data was $\log_2$ transformed and analyzed using CLUSTER and Treeview (Eisen, M. B., Spellman, P. T., Brown, P. O., Botstein, D. (1998) *Proc. Natl. Acad. Sci. U.S.A.* **95**, 14863-14868). Statistical analysis was done using the significance analysis of microarrays algorithm SAM (Tusher, V. G., Tibshirani, R. & Chu, G. (2001) *Proc. Natl. Acad. Sci. U.S.A.* **98**, 5116-5121).

Results:

[0138] The gene expression profiles of 3 populations of dopaminergic neurons (substantia nigra pars compacta (A9 cell group), the ventral tegmental area (A10 cell group) and the zona incerta (A13 cell group)) and one population of noradrenergic cells (locus coeruleus) from adult (7-9 month) female Sprague Dawley rats were analyzed (Figure 2). For each population 3 independent captures of 200 cells from different animals were analyzed and compared to expression of a reference RNA generated from a pool of 3 pooled whole brains from age matched female rats. Genes that showed a statistical significant difference between replicas were identified with SAM (false discovery rate <1%) and subsequently ranked by their average fold expression versus whole brain. Genes with an average expression >8 compared to the whole brain reference were selected (Figures 5-8). To identify genes that confer differential vulnerabilities in Parkinson's disease, genes with significant expression changes between dopaminergic neurons isolated from the substantia nigra and ventral tegmental area (Figures 9 and 10) were selected.

Example 2: Drug Targets Identified In Human Brain Tissue

Tissue

[0139] The Stanford University Medical School Brain Bank provides the brain samples under NIH and Stanford University guidelines. These samples are frozen in liquid nitrogen immediately after dissection. To evaluate the RNA quality of a sample we extract the RNA of a single cyrosection and analyze it on the Agilent bioananlyzer (Fig. 3). In general the degree of preservation of RNA in post mortem human brain

samples is often poor (as assessed by the presence of the ribosomal 18S and 28S ribosomal RNA peaks) and does not directly correlate with the post mortem delay. Therefore only a small subset of autopsy material is suited for single cell microarray analysis experiments (Fig 3).

[0140] Dopaminergic neurons in the substantia nigra and noradrenergic neurons in the locus coeruleus were identified by their content of neuromelanin pigmentation (Fig 4). All experimental steps were carried out as described as described in Example 1 except that no immunostaining was applied.

Results:

[0141] The expression profile of dopaminergic neurons isolated from the human substantia nigra compacta and noradrenergic neurons isolated from the locus coeruleus were analyzed. 200 neuromelanin-containing neurons were isolated by laser microdissection (Figure 4). After two rounds of linear amplification, cRNA was used to generate labeled targets that were hybridized to a cDNA microarray containing 13,000 unique human genes. As a reference, whole brain RNA (Clontec) that had been amplified likewise was used. Genes with an average fold expression >8 compared to the whole brain reference in the substantia nigra or the locus coeruleus are shown in Figures 11 and 12.

Validity of the method

[0142] Many previously known marker genes were detected as highly enriched in their respective cell population in rat as well as human profiling experiments, providing a strong validation of the protocol. Dopaminergic neurons use the neurotransmitter dopamine, which is synthesized from the amino acid tyrosine by two enzymes, tyrosine hydroxylase and DOPA decarboxylase. These enzymes are specifically expressed in the catecholaminergic neurons that constitute only a small subset of neurons within the brain. These genes show a dramatic enrichment in all cell populations profiled. In addition, the genes for dopamine transporter and the presynaptic dopamine receptor D2 showed high expression over whole brain in human substantia nigra and locus coeruleus cells (probes corresponding to these genes are not present on the rat cDNA arrays).

[0143] Noradrenergic neurons signal via the neurotransmitter norepinephrine. In addition to tyrosine hydroxylase and DOPA decarboxlase, these neurons express a third enzyme, dopamine Beta -hydroxylase, that converts dopamine to norepinephrine. This enzyme is exclusively expressed in adrenergic neurons. It was

found that dopamine 3-beta hydroxylase showed the highest expression among genes in purified noradrenergic neurons in humans and rats compared to the whole brain (Figure 12). In addition, tyrosine hydroxylase and DOPA decarboxlase are highly enriched in these cells. Other previously known marker genes for dopaminergic and/or noradrenergic neurons identified in our experiments include e.g. aldehyde dehydrogenase, glutathione peroxidase, gamma-synuclein, and Ret ligand 1 (GFRalpha1).

EXAMPLE 3. Genes that define the four major classes of dopaminergic (DA) and noradrenergic (NA) neurons

Material and method

Tissue preparation and immunohistochemisty

[0144] Brains of adult (7-9 month) female Sprague Dawley rats were dissected and immediately frozen on dry ice. 12 µm cryosections were mounted on polyethylene naphthalene membrane slides pretreated with 0.1% poly-L-lysine for 5 min followed by 30 min of UV irradiation. The sections were fixed immediately in 100% ethanol for 30 s followed by 3 s in acetone and air dried. After rehydration in PBS, pH7.0 for 5 s, the sections were stained for 2 min in PBS, pH7.0; containing 100 µg/ml anti tyrosine hydroxylase antibody (clone TH-16, Sigma) that had been labeled with the Alexa Fluor 488 monoclonal antibody labeling kit (Molecular Probes) according to manufacturer's instructions. Rehydration and staining were performed in the presence of 1U/ul RNAse inhibitor (Roche, Germany). The sections were washed twice in PBS, for 5 s, dehydrated for 30 s in 75%, 95%, and 100% ethanol respectively and air-dried at room temperature.

Laser microdissection, RNA isolation and amplification

[0145] Single neurons were isolated from immunostained cryosections using a PALM Robot-Microbeam system (PALM microlaser technology, Germany). To facilitate detection of fluorescent neurons, a drop of 100% ethanol was applied to the section during cell selection. The sections were allowed to air dry and neurons were dissected and catapulted into 30 µl lysis buffer. Total RNA from 200 pooled neurons was isolated using the Picopure kit (Arcturus) and contaminating genomic DNA was removed during the isolation by an on-column DNAse digestion step. The common reference RNA was generated from 3 pooled whole brains of age matched female rats. RNA was isolated using RNA-Bee (Tel-Test) followed by DNAse digestion with the

DNA-free kit (Ambion). The RNA from dissected neurons and the common reference were amplified by two rounds of T7-based linear amplification (Van Gelder et al. (1990) Proc. Natl. Acad. Sci. USA 87(5):1663-7) using the Riboamp kit (Arcturus) with the following modifications: To minimize generation of template-independent amplification product from the T7 primer, a 1:5 dilution of primer A was used for first round cDNA synthesis and the reaction volume was scaled down by 50%. The yield and size distribution of the amplified RNA product was evaluated by microfluidic gel electrophoresis with the bioanalyzer (Agilent).

RNA labeling, microarray hybridization and data analysis

[0146] Detailed protocols for probe synthesis and DNA microarray hybridization are available at http://cmgm.stanford.edu/pbrown/protocols/index.html. In short, 2 μg of amplified RNA was random primed to generate single-stranded aminoallyl-dUTP cDNA targets, which were subsequently coupled with either Cy3 (whole brain reference) or Cy5 (experimental sample). Experimental and reference samples were combined and hybridized for 48 h at 65°C in deposition hybridization buffer (Agilent) containing 15 μg of each Cot1 DNA, (Invitrogen), yeast t-RNA and polyA (Sigma) and 2.5 μl deposition control targets (Operon) to 14,815-element rat cDNA microarrays (Agilent, G2565A). Microarrays were washed for 5 min in 0.5x SSC, 0.01% SDS, followed by 3 washes in 0.06x SSC for 3 min and scanned on an Agilent G2565AA microarray scanner. Images were analyzed using Agilent feature extraction software (version A.6.1.1). Processing included local background subtraction and a rank consistency-based probe selection for LOWESS normalization. The data was filtered with respect to signal significance. A two tailed t-test was used to determine significance of the signal versus background and spots with a p-value >0.01 in the red or green channel were omitted. Data was $\log_2$ transformed and analysed using Cluster and Treeview (Eisen, M. B. et al (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 14863-14868). Statistical analysis was done using various functions of the significance analysis of microarrays algorithm SAM (Tusher, V. G., et al. (2001) Proc. Natl. Acad. Sci. U.S.A. 98:5116-5121), with a false discovery rate set to < 1%. Only genes for which information was available for more than 80% of arrays were included in the analysis. Four independent experiments were conducted for every cell type and in each experiment cells were isolated from different animals and RNA extraction, amplification, labeling and hybridization were carried out separately. The

mean correlation coefficient of the expression ratios (log2 isolated neurons / whole brain) between replicates was 0.86 and values ranged from 0.81 to 0.93.

In situ hybridization

[0147] Probe templates were amplified from rat brain RNA by nested RT-PCR and T3 promoter sequences were incorporated. The sequence confirmed PCR products were used as templates for synthesis of digoxigenin labeled RNA probes. 20 µm cryosections of rat brain tissue were dried for 30 min at and fixed in 4% paraformaldehyde for 15 min. The sections were bleached in 6% H_2O_2 for 10min, digested with 1µg/ml proteinase K in PBS for 5min and refixed in 4% paraformaldehyde followed by a 10min acetylation step in 0.25% acetic anhydride/100mM Tris pH7.5 and two washes in 2xSSC pH5. The sections were prehybridized in hybridization buffer (5x SSC pH5, 1% blocking reagent (Roche), 50% formamide, 5mM EDTA, 0.1% Tween 20, 10% dextrane sulfate, 100µg/ml salmon sperm DNA, 100µg/ml tRNA, 100µg/ml heparine) for 1h at 65°C and hybridized o/n at 65°C in 100µl hybridization buffer containing 1µg/ml digoxigenin-labeled probe. The slides were washed at 60°C 2x 10 min in 5xSSC, 50% formamide, 2x 15 min in 1xSSC and 30 min 0.2x SSC. DIG epitopes were detected with alkaline phosphatase-coupled anti-digoxigenin Fab fragments (Roche) and developed with BM purple (Roche).

Results

Validation of the experimental approach

[0148] The expression patterns of known key enzymes involved in dopamine and noradrenalin biosynthesis and vesicular transport were used to validate the approach. TH, the rate-limiting enzyme for the synthesis of both dopamine and noradrenalin, was the most highly enriched transcript in all three dopaminergic neuron groups examined (>150 fold compared to the whole brain reference) and the second highest in the noradrenergic neurons. In contrast, dopamine-beta hydroxylase (DBH), which catalyzes the conversion of dopamine to noradrenalin, was exclusively enriched in LC neurons (>500 fold over reference). Other catecholamine synthesis enzymes like the aromatic amino acid decarboxylase (AADC), GTP cyclohydrolase I (GTPCH I) and pterin-4-alpha carbinolamine dehydratase (PCD) and the vesicular monoamine transporter 2 (VMAT-2), which mediate the transport of monoamine neurotransmitters into synaptic vesicles, were also expressed at high levels in all

catecholaminergic neuron populations. As expected, the ubiquitously expressed dihydropteridine reductase (DHPR) did not show significant enrichment in either cell population.

Lineage relationships between catecholaminergic neuronal subclasses

[0149] Lineage relationships between the different classes of catecholaminergic neurons (CA) were determined based on the overlapping patterns of gene expression. Unsupervised hierarchical clustering (Eisen, M. B. et al (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 14863-14868) was used to group the four catecholaminergic neuronal classes based on all the genes represented on the array. Independent gene expression profiles from a given cell group always clustered together, indicating the existence of specific transcriptomes in each subgroup of catecholaminergic neurons. The SN and VTA dopaminergic neurons displayed highly similar signatures of gene expression, suggesting that these anatomically adjacent cell groups are closely related at the molecular level and possibly by lineage. In contrast, the profile of incerto-hypothalamic dopaminergic neurons was only distantly related to those of the SN and VTA neurons, despite the fact that all three groups of neurons use the same transmitter. In fact, in a phylogenetic tree, hypothalamic A13 dopaminergic neurons are not significantly closer to midbrain dopaminergic neurons (DA) than the noradrenergic neurons are.

[0150] Transcripts with at least 4-fold higher expression in any one of the four cell groups compared to whole brain were further studied. Noradrenergic neurons in the LC express the highest number of specifically enriched transcripts (412) followed by SN (279) and VTA (264). Hypothalamic dopaminergic neurons expressed only 170 enriched transcripts. Of the 700 enriched genes only 44 were shared by all four catecholaminergic groups examined. Neurons of the SN and VTA shared the highest number of expressed genes. Of the 372 genes that were expressed at higher levels in either SN or VTA, 46% (171/372) were enriched in both groups of neurons. In contrast, SN and A13 neurons shared 18% (68/381), SN and LC 22% (126/565) and A13 and LC 17% (85/497) of their enriched transcripts.

[0151] In an alternative approach to assess the molecular phylogeny, the percentage of transcripts with differences between any two given cell groups were determined by significance analysis. SN and VTA differed in only 122 (<1%) of their expressed genes. In contrast there were 766 (>5%) differentially expressed transcripts between SN and A13 and 1079 (>7%) between SN and LC neurons. The highest

number of genes with differential expression was observed between LC and A13 neurons (1453; >10%). Taken together, these findings demonstrate that each group of catecholaminergic neurons displays a unique set of expressed genes and support the hypothesis that SN and VTA neurons are closely related by lineage and/or function.

Transcripts enriched in all catecholaminergic neurons

[0152] Transcripts that are expressed at least 4 fold higher in all catecholaminergic neurons than in whole brain were examined (Figure 13). In addition to the expected genes involved in neurotransmitter synthesis and transport such as TH, AADC, GTPCH I, PCD and VMAT-2, the most prominent functional class were genes that counteract stress-induced cell damage. One representative in this group was the transcript coding for glutathione peroxidase, which detoxifies hydrogen peroxide using reduced glutathione. Another gene involved in preventing stress-induced damage was the 8-oxo-dGTPase MTH1, which encodes the key enzyme that counteracts oxidative stress-induced DNA damage by hydrolyzing 8-OxoGTP. Other transcripts with an enriched expression include the caspase recruitment domain-containing molecule ARC, which is a potent repressor of apoptosis and protects cells from hypoxia and oxidative stress (Neuss M. et al. (2001) J. Biol. Chem. 276:33915-22), and the oxygen-regulated protein ORP150, which is induced by hypoxia and excitatory stress and can suppress neuronal death induced by glutamate or ischemia (Tamatani M. et al. (2001) Nat. Med. 7(3):317-23).

[0153] A subset of the transcripts that are expressed more than 4 fold higher (Figure 13 provides additional examples) in all catecholaminergic neurons than in the whole brain are provided in Table 1.

Table 1. Examples of targets expressed >4 fold higher in all catecholaminergic neurons

Gene name	GB accession	Human Orthologs	Unigene	RatioSN	Ratio VTA	Ratio A13	Ratio LC
argininosuccinate synthetase	M31690	NM_054012	Mm.3217	6.0	8.6	11.1	11.6
decay accelerating factor (DAF)	AB032395	M30142	Rn.18841	12.7	14.0	9.2	8.6
MHC class I heavy chain	X90374	U64801	Rn.39743	13.7	11.8	9.0	9.0
cell growth regulator 11	U66470	NM_006569	Rn.31842	10.0	7.5	5.2	11.0
calcyon	AAF34714	NM_015722	Rn.27756	6.4	8.4	7.4	8.4
CLIC3	AAD16450	NM_053603	Rn.1838	5.3	7.2	5.9	5.2
arginine methyltransferase (PRMT2)	AF169620	NM_133182	Mm.32020	6.8	7.8	8.2	9.9

HYPOTHETICAL 38.5 kDA PROTEIN	AK078264	BC047054	Mm.72979	24.5	29.3	6.5	11.3

[0154] The potential stress promoting enzyme argininosuccinate synthetase was highly expressed in all four catecholaminergic neuronal groups. This arginine regenerating enzyme is essential for sustained production of nitric oxide. An excess of nitric oxide has been shown to be neurotoxic while inhibition of NO-synthesis has a neuro-protective effect in the MPTP model of PD (Hantraye P. et al. (1996) Nat. Med. 2(9):1017-21). Two inflammation related genes, decay accelerating factor (DAF), which can protect cells against complement-mediated damage, and MHC class I heavy chain (Linda et al. (1999) J Neuroimmunol. 101(1):76-86), showed a high uniform expression in all cell groups. These molecules could play a role in the neuroinflammatory processes believed to contribute to the degeneration of catecholaminergic neurons in PD.

[0155] Transcripts for Neural Cell Adhesion Molecule (NCAM) along with polysialyltransferase 1, which catalyzes the addition of polysialac acid chains to NCAM and modulates its adhesive properties, were also enriched in all catecholaminergic neurons. Expression of PSA-NCAM, which is involved in the regulation of myelination as well as cell migration, axonal guidance and plasticity, is progressively lost by most tissues during development but appears to be retained in all adult catecholaminergic neuronal classes. Two genes that are associated with modulation of dopamine receptor activity, Calcyon and CLIC3 were also detected in all catecholaminergic neuronal classes. Calcyon is a cross-talk accessory protein which enables the typically Gs-linked D1/D5 dopamine receptor to stimulate intracellular calcium release (Lezcano et al. (2000) Science 287(5458):1660-4). CLIC3 belongs to the family of intracellular choride channels that are involved in a variety of cellular events including secretion, cell division and apoptosis. Another member of this family, CLIC6 has recently been shown to interact with dopamine D2-like receptors (Griffon N. et al. (2003) Brain Res. Mol. Brian Res. 117(1):47-57). The cell growth regulator CGR11 is novel EF-hand domain proteins which is induced by p53 and has been shown to inhibit the growth of several cell lines. The function of the arginine methyltransferase PRMT2 is not known. Post-translational modification of proteins by arginine methylation has recently been implicated in a variety of cellular processes including nuclear receptor transcriptional regulation. The function of the

hypothetical 38.5kDA protein is not known. Is situ hybridization with a probe specific for this transcript confirmed highly specific expression of this gene that is confined to catecholaminergic neurons (Figure 16).

The shared signature of midbrain dopaminergic neurons

[0156] Gene filtering and multiclass significance analysis (Tusher, V. G., et al. (2001) Proc. Natl. Acad. Sci. U.S.A. 98:5116-5121) revealed a set of genes that differ in expression between at least two groups of catecholaminergic neurons (Figure 14). The genes and experimental samples were grouped based on similarities of gene expression by supervised two-dimensional hierarchical clustering. This analysis again demonstrated the high degree of similarity between SN and VTA dopaminergic neurons, and the distant relationship of the SN/VTA with both A13 dopaminergic and LC neurons.

[0157] The SN/VTA cluster consisted of genes with enriched expression in both SN and VTA neurons. Aldehyde dehydrogenase 1 (ALDH1A1), which is known to be highly and specifically expressed in these neurons, served as a validating marker for this gene cluster (Galter et al. (2003) Neurobiol Dis. 14(3):637-47). The cluster contained a large number of transcriptional regulators, including the zinc finger-homeodomain proteins ZFH-4 and ATBF1 (Ishii et al. (2003) J. Comp. Neurol. 465(1):57-71; Kostrich et al. (1995) Dev. Dyn. 202(2):145-52), the homeobox factor PBX1, the forkhead-domain family member FOXP2, the interferon-inducible protein IFI 16 and the matrix attachment region binding protein SATB1. Mutations in FOXP2 are linked to severe speech disorders involving the basal ganglia (Liegeois et al. (2003) Nat. Neurosci. 6(11):1230-7). IFI 16 functions as a transcriptional repressor while SATB1 is a modulator of chromatin (Cai et al. (2003) Nat. Genet. 34(1):42-51). A group of regulators of synaptic signaling and/or plasticity included Synaptotagmin I, the calcineurin inhibitor ZAKI-4, the kinesin related protein Hash, the calcium-activated protein for secretion (CAPS), which controls Ca2+-regulated vesicular exocytosis and the glutamate receptor-interacting protein 2 (Grip2) which is involved in the synaptic targeting of AMPA receptors.

[0158] A subset of genes that are specifically enriched in SN and VTA (Figure 14 provides additional examples of genes and gene clusters) are provided in Table 2.

Table 2. Examples of targets specifically enriched in SN and VTA neurons

Gene name	GB	Human	Unigene	RatioSN	Ratio	Ratio	Ratio

	accession	Orthologs			VTA	Ai3	LC
Caspase 7	Y13088	NM_001227	Mm.35687	11.5	10.3	0.5	0.7
p53 apoptosis associated target (Perp)	AF249870	NM_022121	Mm.28209	13.1	9.4	0.7	4.5
G-protein coupled thrombin receptor	M81642	NM_001992	Rn.2609	8.4	15.7	1.5	1.7
Tumor associated Ca^{2+} signal transducer 1	AJ001044	NM_002354	Rn.24930	9.2	5.1	0.7	0.3
Ca2+-dependent activator protein for secretion 2	BC023929	NM_017954	Mm.259632	10.5	9.7	0.8	0.2

[0159] Three apoptosis related transcripts, Caspase 7, Perp, and SM-20 were highly enriched in SN and VTA neurons. Perp is a positive effector of p53-induced neuronal apoptosis. Moderate levels of Perp were also observed in the LC, while expression was low in hypothalamic dopaminergic neurons which do not degenerate in PD. SM-20 is a mitochondrial protein that promotes caspase-dependent cell death in neurons.

[0160] The protease-activated receptor-1 (PAR-1) is a G-protein coupled receptor that is activated by thrombin. Thrombin has been shown to changes the morphology of neurons and astrocytes, and can have cytoprotective or cytotoxic effects on neural cells. The tumor associated Ca^{2+} signal transducer 1 is a cell surface glycoprotein with unknown function that is highly expressed on most human gastrointestinal carcinoma and at a lower level on most normal epithelia but has not been described in brain tissues. Ca2+-dependent activator protein for secretion 2 is a homolog of the CAPS1 protein which is an essential component of the protein machinery involved in large dense-core vesicle exocytosis and in the secretion of a subset of neurotransmitters.

Transcripts defining LC noradrenergic neurons

[0161] The LC cluster contained the largest collection of cell group specific transcripts (Figure 14). Marker genes for this cluster included DBH, monoamine oxidase A and Cytochrome b561, a major transmembrane protein of catecholamine secretory vesicles that provides reducing equivalents for the DBH reaction. AP-2β, a member of the AP-2 family of retinoic acid-induced transcription factors was highly

enriched in LC neurons. The closely related AP-2α, which recognizes the same target sequence and shares a highly conserved DNA-binding and dimerization domain, has been shown to activate the expression of TH and DBH (Kim et al. (2001) J. Neurochem. 76(1):280-94) and to be essential for the development of noradrenergic LC neurons in zebrafish (Holzschuh et al. (2003) Development 130(23):5741-54).

[0162] A subset of targets with high specific expression in LC noradrenergic neurons (see Figure 14 for additional examples) are provided in Table 3.

Table 3. Example of targets with specific expression in LC noragrenergic neurons

Gene name	GB accession	Human Orthologs	Unigene	RatioSN	Ratio VTA	Ratio A13	Ratio LC
copper transporter 1	AF268030	NM_001859	Rn.2789	2.0	2.3	1.7	39.1
gamma-glutamyltranspeptidase-related enzyme	U76252	NM_004121	Rn.44367	0.4	0.8	1.2	10.8
prostaglandin E synthase	AB041998	NM_004878	Rn.7730	0.5	1.0	1.2	4.0
pigment epithelium-derived factor	AF036164	NM_002615	Mm.2044	0.1	0.2	0.2	12.7

[0163] High specific expressions of the copper transporter 1 and the glutathione metabolizing gamma-glutamyltranspeptidase-related enzyme were observed. Copper is an essential cofactor for various enzymes including Cu, Zn superoxide dismutase, cytochrome oxidase and DBH. However, excess of copper combined with glutathione metabolites leads to free radical damage and possible neuronal dysfunction (Enoiu et al. (2000) Free Radic Biol. Med. 29(9):825-33). The expression of the copper transporter 1 in locus coeruleus neurons further supports the view that metal ion transporters play an important role in determining the vulnerability of neuronal populations to neurotoxic stress. Another potential vulnerability factor in LC neurons was prostaglandin E synthase. LC neurons also expressed high levels of pigment epithelium-derived factor (PEDF), a member of the serine protease inhibitor (serpin) family, which is a survival factor for various types of neurons.

[0164] Other transcripts that are specifically expressed in LC neurons included the oxygen-binding hemoprotein neuroglobin, the inhibitor of apoptosis proteins (IAPs) and the Tumor Necrosis Factor (TNF) receptor associated factor (TRAF) that mediate the anti-apoptotic signals from TNF. A moderate expression of tryptophan hydroxylase in the noradrenergic neurons of the LC, which has been

reported previously (Iijima et al. (1993) Histol Histopathol. 8(3):1387-401) was also observed.

Transcripts defining hypothalamic A13 dopaminergic neurons

[0165] The A13 dopaminergic neurons were characterized by high and specific expression of multiple transcriptional regulators (Figure 14). These included the onecut transcription factor Hnf-6, the LIM-only protein Lmo2, the zinc finger Bteb2 and the homeodomain proteins Isl-1, Nkx2.1, Dlx, Six3, Lim1, Prox1 and Arx. Six3 has been shown to alter the regional responses to Fgf8 and Shh, which is required for development of the hypothalamus (Kimura et al. (1996) Genes Dev. 10(1):60-9). The Arx, Dlx, Isl-1, Lim1 and Nkx2.1 are important regulators of proliferation, migration and differentiation of neurons in the embryonic forebrain (Kitamura et al. (2002) Nat. Genet. 32(3):359-69). In Dlx1/2 mutants for example, the A13 dopaminergic neurons do not form (Andrews et al. (2003)). The functions of Hnf-6, Lmo2, Bteb2, and Prox1 in the A13 dopaminergic neurons are currently not known. The fact that expression of multiple developmental regulators is sustained in adult rat brains, suggests additional yet-to-be identified functions.

[0166] Like the SN/VTA neurons, the A13 dopaminergic neurons and the noradrenergic neurons in the LC each also expressed their own characteristic member of the aldehyde dehydrogenase family. The ALDH1A3 family member expressed in the hypothalamus and ALDH1A1 in the SN/VTA can both convert retinaldehyde to retinoic acid. Signaling of retinoic acid was shown to be involved in many developmental processes including the specification of motorneurons sub-classes (Sockanathan and Jessell (1998) Cell 94(4):503-14) and might also be important in the catecholaminergic system. ALDH3A1 expressed in LC neurons is not capable of synthesizing retinoic acid but could be involved in detoxification and the metabolism of neurotransmitters.

Differential gene expression in SN and VTA neurons

[0167] Transcripts which are differentially expressed between SN and VTA neurons were identified by two-class significance analysis (Figure 15). Among these were transcripts from various functional categories including transcriptional regulators (Sox-6, Zfp 288, HTF, NGFI-A), molecules involved in vesicle trafficking (DOC2B, rab3B, MARCKS), axon guidance (neuropilin-1, Slit-2 and Ephrin B3), transporters (VGLUT2, CNT2) and ion channels (CLIC5).

[0168] The most prominent gene classes identified encoded factors involved in cell survival and protection which were all expressed at a higher level in the VTA neurons. A subset of such transcripts are provided in Table 4 (see also Figure 15).

Table 4. Examples of targets with differential expression in SN and VTA neurons

Gene name	GB accession	Human Orthologs	Unigene	RatioSN	Ratio VTA	Ratio A13	Ratio LC
pituitary adenylate cyclase-activating polypeptide	M63006	NM_001117	Rn.37400	0.2	4.0	0.3	1.4
atrial natriuretic peptide	K02062	NM_006172	Rn.2004	0.5	1.8	14.1	2.9
bone morphogenic protein 2	L20678	NM_001200	Rn.12687	1.0	2.6	0.3	1.4
castration induced prostatic apoptosis-related protein 1	AJ010750	NM_015393.	Rn.21667	1.0	5.0	7.4	1.2
Extracellular superoxide dismutase	Z24721	NM_003102	Rn.10358	0.5	1.2	0.4	0.4
Lipoprotein lipase	L03294	NM_000237	Rn.3834	0.1	2.0	0.1	0.0
UDP-glucuronyltransferase-S.	AB010441	NM_080742	Rn.42869	0.6	2.6	2.5	1.2
GPRC5A	NM_181444	NM_003979	Mm.23575	7.8	2.0	2.0	1.9
Zn^{2+} transporter ZIP-4	BQ196656	NM_017767	Rn.7960	103.6	265.5	1.6	1.4
gamma-synuclein	X86789	NM_003087	Rn.10421	28.1	4.3	1.8	29.5
protein kinase C delta	M18330	NM_006254	Rn.98279	0.9	0.2	0.1	0.1

[0169] PACAP and BMP-2 are known survival factors for ventral mesencephalic dopaminergic neurons that can protect from 6-hydroxydopamine and MPTP (Espejo et al., (1999) Neurosci Lett. 275(1):13-6; Reiriz et al. (1999) J. Neurobiol. 38(2):161-70; Takei et al. (1998) J. Neurosci Res. 54(5):698-706). ANP can counteract oxidative stress and excess NO (Vaudry et al. (2002) Eur. J. Neurosci. 15(9):1451-60; Fiscus (2003) Neurosignals 11(4):175-90). PARM-1 is implicated in suppression of apoptosis (Bruyninx et al. (1999) Endocrinology 140(10):4789-99). The expression BMP-2 was paralleled by the BMP-inducible antagonists follistatin and chordin, which is indicative of active BMP-signaling in adult VTA neurons controlled by autoregulatory feedback loops.

[0170] Enriched expression in VTA over SN neurons was also observed for enzymes with detoxifying properties. See Table 4 and Figure 15. Extracellular

superoxide dismutase is an antioxidant enzyme that attenuates brain and lung injury from oxidative stress (Sheng et al. (2000) Exp. Neurol. 163(2):392-8). Lipoprotein lipase is a key enzyme involved in the metabolism of lipoproteins, which protects from cell death induced by oxidized lipoproteins (Paradis et al. (2003) J. Biol. Chem. 278(11):9698-705). UDP-glucuronosyltransferase detoxifies compounds by conjugation to glucuronic acid. On the other hand, expression of PKC-delta, a potent promoter of neurodegeneration, was significantly lower in VTA neurons compared to the SN. Proteolytic activation of PKC delta has been shown to mediate dopaminergic neuronal cell apoptosis induced by MPTP or pesticides (Kaur et al. (2003) Neuron 37:899-909; Kitazawa et al. (2003) Neuroscience 119(4):954-64). High expression of gamma-synuclein in neurons of the SN compared to the VTA was also observed. gamma-synuclein transcripts are highly enriched in both, SN and LC (28 and 29-fold respectively), which are vulnerable to PD and is dramatically lower in the less vulnerable VTA (4 fold) and A13 (2 fold) neurons and it may contribute to the SN and LC specific toxic effects of the widely expressed α-synuclein protein. Retinoic acid induced 3 (RAI3 or GPRC5A) is an orphan G protein-coupled receptor with unknown function that is induced by retinoic acid. This gene is a member of the type 3 G protein-coupling receptor family, characterized by the signature 7-transmembrane domain motif and may be involved in interaction between retinoid acid and G protein signalling pathways.

[0171] The Zn^{2+} transporter ZIP-4 was also dramatically enriched in the SN (>100 fold) and the VTA (>250 fold) but not in A13 or LC. The specific expression of ZIP-4 was confirmed by in situ hybridization (Fig. 16). Zn^{2+} ions could play a role in the pathophysiology of Parkinson's disease. Metal ions increase oxidative damage following energy failure in the cells. Parkinson research has emphasized Fe^{2+} because of the high concentration of this metal ion in the substantial nigra. Chelators of Fe^{2+} prevent the toxic effects of MPTP on dopaminergic neurons (Kauer l.,(2003) Neuron 37(4):549-50). Zn^{2+} has been the focus of attention in neurodegeneration in the hippocampus following ischemic stroke. The granule cells contain high levels of Zn^{2+} that is released synaptically and able to damage postsynaptic neurons at high concentrations (Sloviter, (1985) Brain Res. 330(1): 150-3). Subsequent studies confirmed the toxicity of Zn^{2+} in cell culture systems., Zn^{2+} can also act as an inhibitor of cell death if present at low concentrations. Based on thefindings presented here

the Zn^{2+} transporter ZIP-4 could have an important role in dopaminergic neurotoxicity and could be useful as a drug target.

[0172] Other transcripts that were highly enriched in SN and VTA neurons include factors with a reported or anticipated function in synaptic plasticity, including the synaptic adhesion molecules synCAM and syndecan-2 (Yamagata (2003) Curr. Opin. Cell Biol. 15(5):621-32) and the actin-associated synaptopodin-2 which belongs to a class of factors required for the formation of the spine apparatus in dendritic spines, an important site of neuronal plasticity (Deller et al. (2003) Proc. Natl. Acad. Sci. USA 100(18):10494-9). The myristoylated alanine-rich C kinase substrate (MARCKS) and G-substrate are substrates of protein kinase C and cGMP-dependent protein kinase respectively and have been implicated in learning and long-term potentiation (LTP). Phospholipase-Cγ (PLCγ) is suspected to be involved in the maintenance of LTP (Ernfors and Bramham (2003) Trends Neurosci. 26(4):171-3) while NGFI-A or Zif268 is an immediate early gene associated with learning and plasticity. The serine proteases, RNK-Met 1 and DISP as well as the serine protease inhibitor Hai2 which might contribute to synaptic plasticity by modulation of the extracellular environment were also identified.

[0173] This study analyzed the molecular signatures that define the major subpopulations of CA neurons. It was shown that individual neurons can be identified by a rapid immunostaining protocol and isolated from brain tissue with an intact complement of RNA that is suited for amplification and microarray analysis. Phylogenetic analysis revealed a very close relationship between midbrain DA neurons in the SN and the VTA. Despite considerable heterogeneity in the mesotelencephalic DA system with respect to cell morphology, target innervation, electrophysiological properties, and disease susceptibility, this study determined differential expression of less than 1% of their genes. In contrast, 5% of the transcripts in the hypothalamic DA neurons differed from these of the SN or VTA neurons. DA neurons in the midbrain and hypothalamus each expressed their own specific sets of transcriptional regulators. This suggests that the DA phenotype in these groups of neurons could be maintained, at least in part, by independent regulatory cascades. In fact, while midbrain and forebrain DA neurons depend on the same signaling molecules (FGF 8 and Shh) during early development, several factors

have been identified that selectively control DA fate in the midbrain (Nurr1, Lmx1b, Pitx3).

[0174] The fourth cell group analyzed, the NA neurons in the LC, displayed differences in transcripts of about 7% when compared to the DA SN or VTA and of more than 10% compared to the hypothalamic A13 cell group. In LC NA neurons, the expression of dopamine synthesizing enzymes seems to be controlled by a different transcription factors than in the DA cell groups (Goridis and Rohrer (2002) *Nat. Rev. Neurosci.* 3(7):531-41). In contrast to DA neurons in the midbrain and hypothalamus, only a single transcription factor, AP-2β, with LC specific expression was identified. The closely related family member AP-2α, which was not present on the array, has recently been shown to activate the TH and DBH promoters (Kim et al. (2001) J. Neurochem. 76(1):280-94) and to be required for the development of LC neurons in zebrafish embryos (Holzschuh at al. (2003) *Development* 130(23):5741-54). The precise role of AP-2β in NA neurons which recognizes the same target sequence and can heterodimerize with AP-2 α is not known. .

[0175] The complexity of cell group specific gene expression seems to be correlated with the diversity of projections and the complexity of biological functions of the individual CA subclasses. The LC NA system, which provides a highly divergent innervation to virtually the entire CNS, allowing it to regulate emotional, cognitive and sleep-wake functions, expressed the highest number of specific genes. In contrast, hypothalamic A13 neurons which have a less extensive network of projections and control less diverse brain functions, expressed less than half that number of specific genes, while SN and VTA had intermediate numbers of enriched transcripts.

[0176] Despite the high similarity of the transcriptomes in SN and VTA neurons, a number of subpopulation-specific genes were identified. Among the gene transcripts enriched in the VTA were several encoding synaptic plasticity proteins such as PLCγ, synCAM, syndecan-2, synaptopodin-2, MARCKS, G-substrate, and Zif268. These could may contribute to the long-term synaptic plasticity elicited by psychostimulants leading to drug addiction (Gerdemann et al. (2003) *Trends Neurosci.* 26(4):184-92). A critical role of PLCγ in the regulation of long-term adaptations to drugs has recently been demonstrated by overexpression experiments in

the VTA (Bolanos et al. (2003) *J. Neurosci.* 23(20):7569-76). Likewise, the expression of the learning and plasticity-associated immediate early gene Zif268 is induced in VTA neurons upon drug-conditioned stimulation and decreases during prolonged withdrawal (Thomas et al. (2003) *Eur. J. Neurosci* 17(9):1964-72; Mutschler et al (2000) *Neuroscience* 100(3):531-8).

[0177] VTA neurons were also enriched in several factors involved in axonal pathfinding and neuronal migration (neuropilin-1, slit-2 and ephrin B3). During development, SN neurons target mainly the dorso-lateral striatum while VTA neurons mainly innervate the ventromedial striatum, constituting mesostriatal and mesolimbic pathways respectively. The molecular signals that regulate the development of these pathways have only been partially characterized (Yue et al. (1999) *J. Neurosci* 19(6):2090-101) and differential expression of multiple members of the ephrin/Eph and slit/robo family identified here could have important functions in path finding and adult plasticity. These findings are particularly interesting from a point of view of schizophrenia, a disease most likely linked to abnormal developmet of cortical areas innervated by the VTA neurons (Lewis and Levitt (2002) *Neurosci.* 25:409-432). DISC1, the first discovered schizophrenia gene, is expressed at highest levels in the cortex during development. It interacts with NudE-like (NUDEL) earlier linked to cortical development (Ozeki et al. (2003) *Proc. Natl. Acad. Sci. USA* 100:289-294). Linkage studies have identified neuregulin 1 as a susceptibility gene in Islandic and Scottish populations (Stefansson et al. (2003) *Am. J. Hum. Genet.* 72:83-87). Neuregulin is a member of a multigene family of transmembrane proteins that contain an extracellular EGF-like domain necessary for function and which play an important role in the developmental of neurons and glial cells. A further schizophrenia susceptibility gene identified by linkage studies is dystrobrevin-binding protein 1, a protein is contained in postsynaptic densities and functionally linked to synaptic plasticity (Straub et al. (2002) *Am. J. Hum. Genet.* 71:337-348). These findings are compatible with the view that schizophrenia is, at least in part, a developmental disorder of the development of the cortex. The genes identified in this study as selectively expressed by VTA DA neurons could participate in the disease-related pathways of schizophrenia.

[0178] A goal of this analysis was to identify genes that may influence the selective vulnerability catecholiminergic (CA) neurons in Parkinson's Disease (PD).

The subpopulation of dopamine neurons confined to the zona compacta of the substantia nigra are most susceptible to Parkinson's disease pathology. Their degeneration causes the vast majority of behavioral symptoms of the disease. The adjacent VTA dopamine neurons are less vulnerable, and hypothalamic DA neurons are spared (Farneley and Lees (1991) *Brain* 114 (Pt 5): 2283-2301; Hirsch et al., (1988) *Nature* 334:345-348; Uhl et al. (1985) *Neurology* 35(8):1215-8; Purba et al. (1994) *Neurology* 44(1):84-9; Matzuk et al., (1985) *Ann Neurol* 5:552-5). The same selective vulnerability of DA neuron subpopulations has been observed in rodent and primate models of PD (Melamed et al. (1985) *Eur. J. Pharmacol.* 114(1):97-100; Mogi et al. (1988) *J. Neurochem.* 50(4):1053-6; Zuddas et al. (1989); Varastet et al. (1993) *Neuroscience* 63(1):47-56). Based on the expression of genes known to counteract stress-induced cell damage (glutathione peroxidase, 8-oxo-dGTPase, ARC, ORP150), it appears that all CA cell groups are under oxidative stress possibly resulting from DA metabolism. The selective vulnerability could reside in the multiple cell group specific transcripts for regulators of oxidative stress, excitotoxicity, apoptosis, mitochondrial dysfunction and neuroinflammation that we have identified. For instance, significance analysis identified a group of VTA-enriched neuroprotective factors including neurotrophic factors (BMP-2, PACAP, ANP), detoxifying enzymes (EC-SOD, lipoprotein lipase, UDP-glucuronosyltransferase), the anti-apoptotic factor PARM-1 and decreased levels of the pro-apoptotic PKC delta that may account for the sparing of VTA neurons in PD. High expression of gamma -synuclein in neurons of the SN and in LC noradrenergic neurons that degenerate in PD compared to the VTA and A13 which could contribute to the SN and LC specific toxic effects of the widely expressed gamma -synuclein protein was also observed..

[0179] The selective expression of the Zn^{2+} transporter by the SN and VTA suggests that the possibility that this ion plays a role in the pathophysiology of Parkinson's disease. Metal ions increase oxidative damage following energy failure in the cells. Parkinson research has emphasized Fe^{2+} because of the high concentration of this metal ion in the substantial nigra. Chelators of Fe^{2+} prevent the toxic effects of MPTP on DA neurons (Kaur et al. (2003) *Neuron* 37:899-909). Zn^{2+} has been the focus of attention in neurodegeneration in the hippocampus following ischemic stroke. The granule cells contain high levels of Zn^{2+} that is released synaptically and

able to damage postsynaptic neurons at high concentrations (Sloviter (1985) *Brain Res.* 330:150-153). Subsequent studies confirmed the toxicity of Zn^{2+} in cell culture systems. The findings suggest that Zn^{2+} could be equally important for DA neurotoxicity. The expression of the copper transporter 1 in locus coeruleus neurons further supports the view that metal ion transporters play an important but complex role in determining the vulnerability of neuronal populations to neurotoxic stress.

[0180] The findings herein, provide the first genomic analysis of clinically relevant classes of CA neurons revealing previously unrecognized patterns of gene expression that are shared or confined to specific populations of CA neurons. The data leads to better understanding of the distinct features and functions of these groups of neurons and provides drug targets that could be useful for drug development. For example, the drug targets presented in Tables 1-4, could be useful for Parkinson's disease.

[0181] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to those skilled in the art that certain changes and modifications may be practiced. Therefore, the descriptions and examples should not be construed as limiting the scope of the invention.

CLAIMS

We claim:

1. A method of identifying a candidate drug target in a population of dopaminergic or noradrenergic neurons comprising evaluating the expression of one or more polynucleotides in a dopaminergic or noradrenergic neuron population, wherein the one or more polynucleotides are candidate drug targets.

2. A method of identifying candidate drug targets in a population of dopaminergic or noradrenergic neurons comprising : (a) identifying the population of neurons, (b) isolating the populations of neurons; (c) evaluating the expression of one or more polynucleotides in the population of neurons, wherein the one or more polynucleotides and/or the one or more encoded polypeptides are candidate drug targets.

3. The method of claim 1 or claim 2, further comprising the step of evaluating the expression of one or more polynucleotides in step (b) relative to a control population of neurons.

4. The method of claim 3, wherein the control population of neurons is from whole brain.

5. The method of claim 1 or claim 2, wherein the population of neurons are dopaminergic neurons.

6. The method of claim1 or claim 2, wherein the population of neurons are noradrenergic neurons.

7. The method of claim 5, wherein the population of dopaminergic neurons are substantia nigra neurons.

8. The method of claim 5, wherein the dopaminergic neurons are ventral tegmental area neurons.

9. The method of claim 5, wherein the dopaminergic neurons are zona encarta (A13 group) neurons.

10. The method of claim 6, wherein the noradrenergic neurons are locus coeruleus neurons.

11. The method of claim 1 or claim 2 , wherein the population of neurons are obtained from a pathology sample, an autopsy sample, a biopsy sample, a brain tissue bank or in vitro cultures of dopaminergic or noradrenergic neurons.

12. The method of any one of claims 1-11, wherein the population of neurons are human neurons or rodent neurons.

13. The method of any one of claims 1-11, wherein the level of expression of the gene transcript corresponding to the drug target is evaluated.

14. The method of any one of claims 1-11, wherein the level of expression of the polypeptide corresponding to the drug target is evaluated.

15. A method of assessing the ability of a candidate agent to modulate dopaminergic or noradrenergic neuron activity and/or function comprising measuring the level of expression of the one or more drug targets selected by the method of claim 1 or claim 2, wherein an alteration of the level of expression of the one or more drug targets indicates the ability of the candidate agent to modulate dopaminergic and/or noradrenergic neuron activity and /or function.

16. A method of assessing the ability of a candidate agent to modulate dopaminergic or noradrenergic neuron activity or function comprising: (a) contacting a population of dopaminergic and/or noradrenergic neurons expressing one or more drug targets with a candidate agent, wherein the one or more drug targets are selected from the drug targets referenced in Figure 5, Figure 6, Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12, Figure 13, Figure 14, Figure 15, Table 1, Table 2, Table 3 or Table 4 or combinations thereof and (b) measuring the level of expression of the one or more drug targets in the population of dopaminergic or noradrenergic

neurons, wherein an alteration of the level of expression of the one or more drug targets indicates the ability of the candidate agent to modulate dopaminergic or noradrenergic neuron activity or function.

17. The method of claim 16, wherein the population of neurons are substania nigra dopaminergic neurons and the one or more drug targets are selected from Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14 Figure 15, Table 1, Table 2 or Table 4 or combinations thereof.

18. The method of claim 16, wherein the population of neurons are zona incerta A13 neurons and the one or more drug targets are selected from Figure 5, Figure 13, Figure 14 or Table 1 or combinations thereof.

19. The method of claim 16, wherein the population of neurons are ventral tegmental area neurons and the one or more drug targets are selected from Figure 7, Figure 9, Figure 10, Figure 13, Figure 14, Figure 15, Table 1, Table 2 or Table 4 or combinations thereof.

20. The method of claim 16, wherein the population of neurons are locus coeruleus neurons and the one or more drug targets are selected from Figure 6, Figure 12, Figure 13, Figure 14, Table 1 or Table 3 or combinations thereof.

21. The method of any one of claims 16-20, wherein the level of expression of the gene transcript corresponding to the drug target is measured.

22. The method of any one of claims 16-20, wherein the level of expression of the polypeptide corresponding to the drug target is measured.

23. A method of assessing the ability of a candidate agent to modulate dopaminergic or noradrenergic neuron activity and/or function comprising: (a) contacting a population of dopaminergic and/or noradrenergic neurons expressing one or more drug targets with a candidate agent, wherein the one or more drug targets are selected from the drug targets referenced in Table 1, Table 2, Table 3 or Table 4 and (b) measuring the level of expression of the one or more drug targets in the population

of dopaminergic and/or noradrenergic neurons, wherein an alteration of the level of expression of the one or more drug targets indicates the ability of the candidate agent to modulate dopaminergic or noradrenergic neuron activity and/or function.

24. The method of claim 23, wherein the one or more drug targets are selected from Table 1.

25. The method of claim 23, wherein the population of neurons are substania nigra dopaminergic neurons and the one or more drug targets are selected from Table 2 or Table 4 or combinations thereof.

26. The method of claim 23, wherein the population of neurons are ventral tegmental area neurons and the one or more drug targets are selected from Table 2 or Table 4 or combinations thereof.

27. The method of claim 23, wherein the population of neurons are locus coeruleus neurons and the one or more drug targets are selected from Table 3.

28. The method of claim 23, wherein the population of neurons are locus coeruleus neurons and the one or more drug targets are selected from Table 1 and 3.

29. The method of claim 23, wherein the population of neurons are zona incerta A13 neuron neurons and the one or more drug targets are selected from Table 1.

30. The method of any one of claims 23-29, wherein the level of expression of the gene transcript corresponding to the drug target is measured.

31. The method of any one of claims 23-29, wherein the level of expression of the polypeptide corresponding to the drug target is measured.

32. A method of assessing the ability of a candidate agent to bind to one or more drug targets for dopaminergic or noradrenergic neurons, said method comprising : (a) contacting the one or more drug targets selected from Figure 5,

Figure 6, Figure 7, Figure 8, Figure 9, Figure 10, Figure 11, Figure 12, Figure 13, Figure 14, Figure 15, Table 1, Table 2, Table 3 or Table 4 or combinations thereof with a candidate agent and (b) evaluating the binding of the candidate agent to the drug target, wherein the ability of the candidate agent to bind to the drug target is indicative of the possible therapeutic potential of the candidate agent.

33. The method of claim 32, wherein the neurons are substania nigra dopaminergic neurons and the one or more drug targets are selected from Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14 Figure 15, Table 1, Table 2 or Table 4 or combinations thereof.

34. The method of claim 32, wherein the neurons are zona incerta A13 neurons and the one or more drug targets are selected from Figure 5, Figure 13, Figure 14 or Table 1 or combinations thereof.

35. The method of claim 32, wherein the neurons are ventral tegmental area neurons and the one or more drug targets are selected from Figure 7, Figure 9, Figure 10, Figure 13, Figure 14, Figure 15, Table 1, Table 2 or Table 4 or combinations thereof.

36. The method of claim 32, wherein the neurons are locus coeruleus neurons and the one or more drug targets are selected from Figure 6, Figure 12, Figure 13, Figure 14, Table 1 or Table 3 or combinations thereof.

37. The method of claim 32, wherein the drug target is a polynucleotide drug target.

38. The method of claim 32, wherein the drug target is a polypeptide.

39. The method of claim 32, wherein the level of expression of the gene transcript corresponding to the drug target is measured.

40. The method of claim 32, wherein the level of expression of the polypeptide corresponding to the drug target is measured.

41. The method of any one of claims 32-40, wherein the candidate agent is an antibody.

42. A microarray comprising polynucleotide drug targets for substantia nigra neurons, the microarray comprising one or more of the polynucleotide drug targets or fragments thereof referenced in Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14, Figure 15, Table 1, Table 2 or Table 4.

43. A microarray comprising polynucleotide drug targets for zona incerta A13 neurons, the microarray comprising one or more drug targets are selected from Figure 5, Figure 13, Figure 14 or Table 1.

44. A microarray comprising polynucleotide drug targets for ventral tegmental area neurons, the microarray comprising one or more of the polynucleotide drug targets or fragments thereof referenced in Figure 7, Figure 9, Figure 10, Figure 13, Figure 14, Figure 15, Table 1, Table 2 or Table or 4.

45. A microarray comprising polynucleotide drug targets for locus coeruleus neurons, the microarray comprising one or more of the polynucleotide drug targets or fragments thereof referenced in Figure 6, Figure 12, Figure 13, Figure 14, Table 1 or Table 3.

46. A microarray comprising at least 2, 3, 5, 10, 20, 40, 50, 60, 70, 80, 90, 100, 200, 300 or 400 of the polynucleotide drug targets in claims 42-45 or combinations thereof.

47. A microarray comprising at least 2, 3, 5, 10, 20, 40, 50, 60, 70, 80, 90, 100, 200, 300 or 400 of the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4.

48. The microarray of claim 47 comprising at least 20, 30, 40 or 50 of the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4.

49. The microarray of claim 47 comprising at least 60, 70, 80, 90, 100, 200, 300 or 400 of the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4.

50. A microarray comprising polypeptide drug targets for substantia nigra neurons, the microarray comprising one or more of the polypetide drug targets or fragments thereof encoded by a polynucleotide referenced in Figure 8, Figure 9, Figure 10, Figure 11, Figure 13, Figure 14, Figure 15, Table 1, Table 2 or Table 4.

51. A microarray comprising polypeptide drug targets for zona incerta A13 neurons, the microarray comprising one or more of the one or more of the polypetide drug targets or fragments thereof encoded by a polynucleotide referenced in Figure 5, Figure 13, Figure 14 or Table 1.

52. A microarray comprising polypeptide drug targets for ventral tegmental area neurons, the microarray comprising one or more of the polypetide drug targets or fragments thereof encoded by a polynucleotide referenced in Figure 7, Figure 9, Figure 10, Figure 13, Figure 14, Figure 15, Table 1, Table 2 or Table or 4.

53. A microarray comprising polypeptide drug targets for locus coeruleus neurons, the microarray comprising one or more of the polypetide drug targets or fragments thereof encoded by a polynucleotide referenced in Figure 6, Figure 12, Figure 13, Figure 14, Table 1 or Table 3.

54. A microarray comprising at least 2, 3, 5, 10, 20, 40, 50, 60, 70, 80, 90, 100, 200, 300 or 400 of the polypeptide drug targets in claims 50-53 or combinations thereof.

55. A microarray comprising at least 2, 3, 5, 10, 20, 40, 50, 60, 70, 80, 90, 100, 200, 300 or 400 of the polypeptide drug targets encoded by the polynucleotide drug targets referenced in Figures 5-15 and Tables 1-4.

56. A kit comprising one or more microarrays of any of claims 42-55.

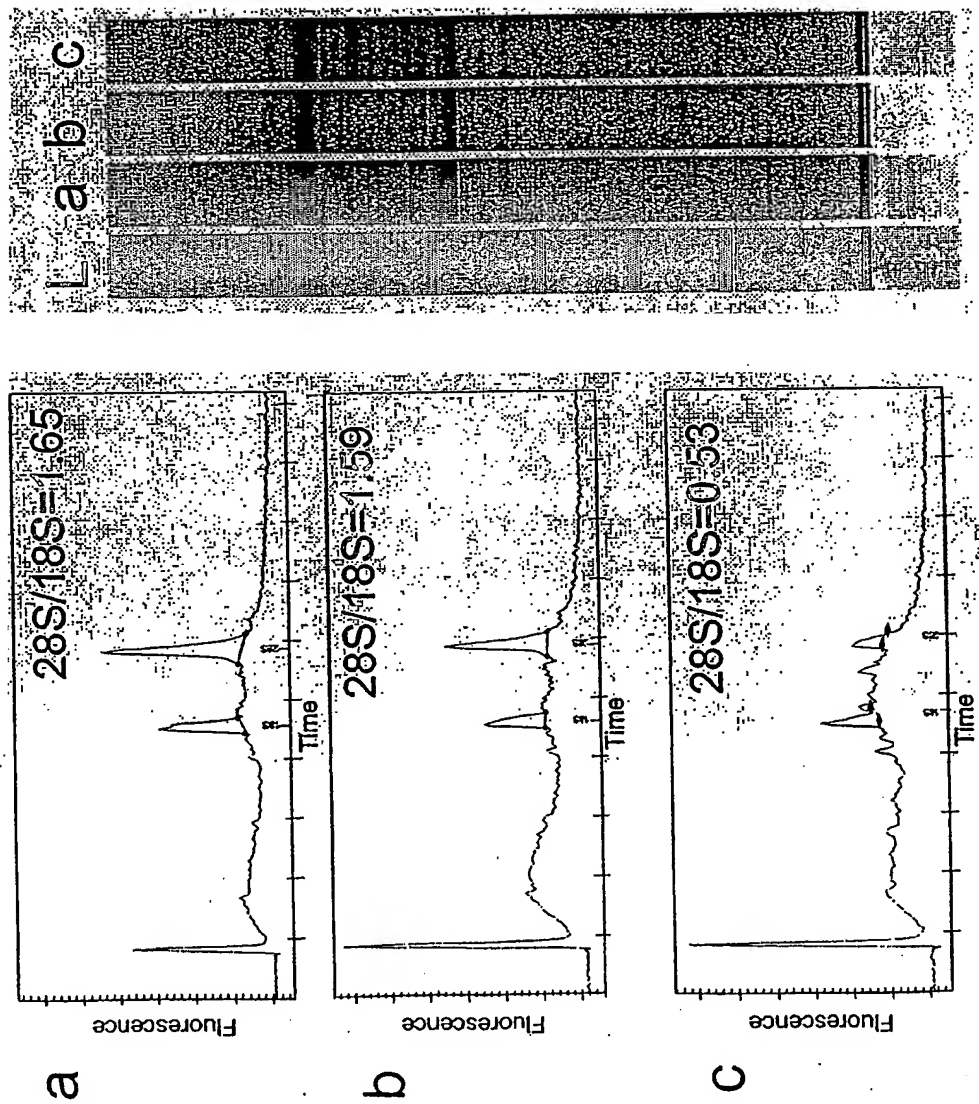

L a b c
28S/18S=1.65
28S/18S=1.59
28S/18S=0.53
Time
Fluorescence
a
b
c

Figure 1

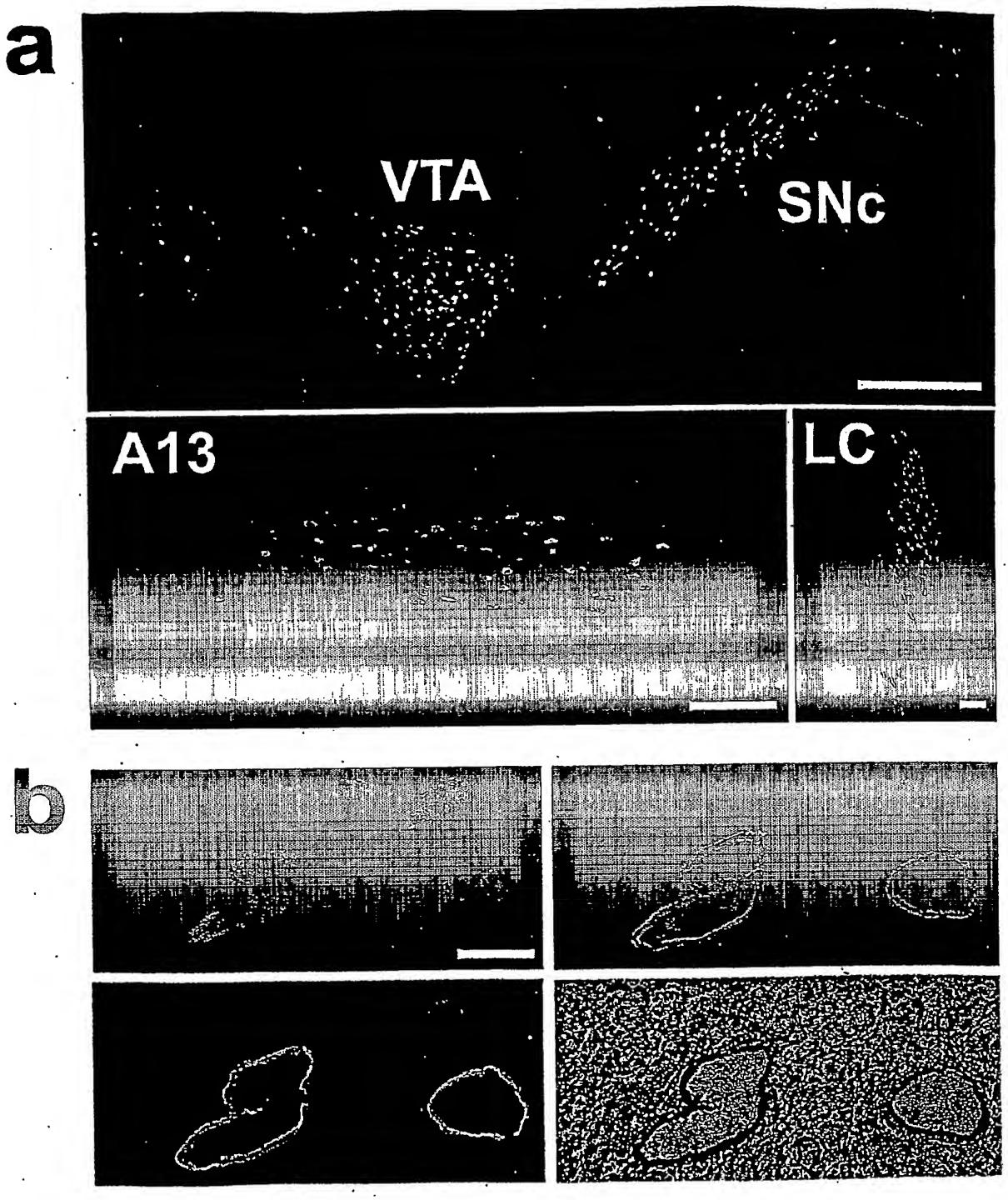


Figure 2

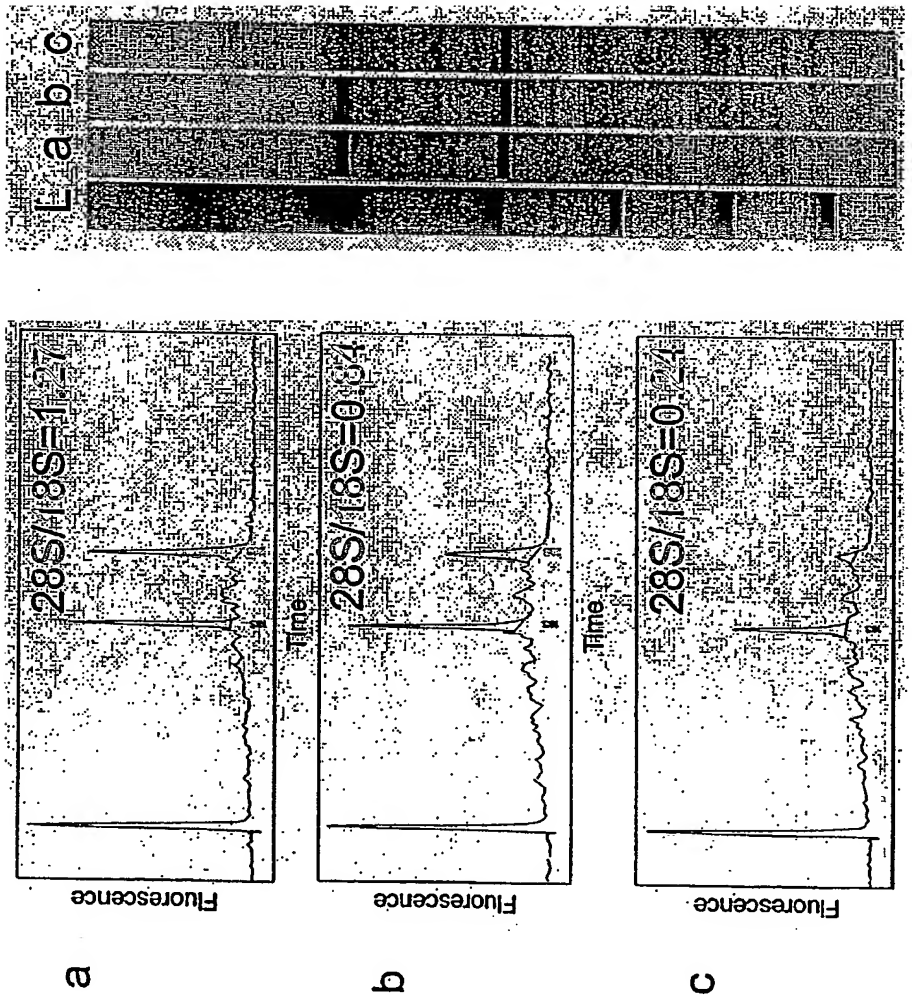

L a b c
28S/18S=1.27
28S/18S=0.34
28S/18S=0.24
Time
Fluorescence
a
b
c

Figure 3

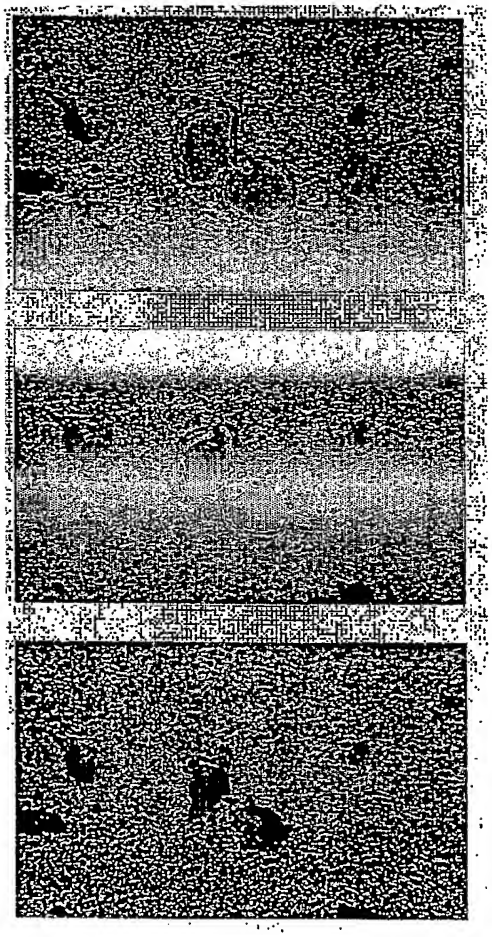


Figure 4

A13 GENELIST RAT

GENE NAME	GB ACCESSION	HUMAN ORTHOLOGS	UNIGENE	RATIO
Rat tyrosine hydroxylase mRNA, complete cds.	M10244	THC1240552	Rn.11082	149.7
Rat (Sprague-Dawley) mRNA for growth hormone-releasing hormone -like peptide.	Z34092	NM_021081 X00094	0	133.6
Rattus norvegicus cDNA clone UI-R-C4-sly-h-12-0-UI similar to HNF6 Hepatocyte nuclear factor 6 (HNF-6)	AW535616	BM682493	Mm.227132	89.1
reserpine-sensitive vesicular monoamine transporter	A46374	THC1234153	Mm.91919	67.6
Rat dopa decarboxylase (DDC) mRNA, complete cds.	M27716	THC1346419 THC1346420	Rn.11064	40.7
UI-R-BS2 Rattus norvegicus cDNA clone	BF396492	BM679470	Rn.58613	40.0
Rat RET ligand 1 (RETL1) mRNA, complete cds.	U97142	THC1301102	Rn.6281	34.0
Rat mRNA for glucokinase, alternatively spliced GK2 (EC 2.7.1.1).	X53586	THC1307452	Rn.10447	32.7
Rattus norvegicus cDNA clone UI-R-CV1-brw-a-04-0-UI 3' weakly similar to gamma-aminobutyric acid (GABA-A) receptor, subunit epsilon	BG373206	N.A.	Rn.17221	28.2
Rat mRNA for hepatocyte nuclear factor 6 alpha.	X96553	THC1337347	Rn.48812	27.4
GTP cyclohydrolase I (EC 3.5.4.16) precursor - rat.	A39080	THC1286628	Rn.2987	27.0
Rat developmentally regulated intestinal protein (OCI-5) mRNA, complete cds. Glypican 3	M22400	NM_004484	Rn.8717	26.3
Rattus norvegicus cDNA clone UI-R-CX0-bxm-h-07-0-UI 3' moderately similar to Tnf receptor-associated factor 3 (Traf3) gene, partial sequence; and amnionless precursor protein (Amn) gene	BI276908	N.A.	Rn.7767	25.2
Mouse mRNA for Lim-homeodomain protein Islet1.	AJ132765	THC1336524	0	21.4
Mouse mRNA for N-glycan alpha 2,6-sialyltransferase.	X83562	THC1307656	0	20.3
Rat CART protein mRNA, complete cds.	U10071	THC1384552	Rn.10201	19.6
Rat peripherin mRNA, complete cds.	AF031878	THC1397870	Rn.11086	16.6
Rat mRNA for plasma glutathione peroxidase precursor, complete cds.	D00650	THC1406411 THC1406413	Rn.1491	16.3
KIAA0605 protein hypothetical Thrombospondin type I repeat (TSP1) profile/Thrombospondin type I domain containing protein	BAA25531	NM_014694	0	15.2
Rat PND gene encoding atrial natriuretic factor, complete cds.	K02062	THC1305948	Rn.2004	14.1
Rat DCoH gene. Rattus norvegicus 6-pyruvoyl-tetrahydropterin synthase/dimerizationcofactor of hepatocyte nuclear factor 1 alpha (Pcbd), mRNA.	AJ005542	THC1251737	Rn.7223	13.7
Rat PND gene encoding atrial natriuretic factor, complete cds.	K02062	THC1305948	Rn.2004	11.1
Mouse argininosuccinate synthetase (Ass) mRNA, complete cds.	M31690	THC1396032 THC1396033	0	11.1
Rat collagen type II mRNA, complete cds.	L49440	THC1376376 THC1376377 THC1399286 THC1390757 THC1390758	Rn.10124	11.0
Rattus norvegicus cDNA clone UI-R-FJ0-cqb-p-07-0-UI 3' undescribable transcript, int insert sequence	CA506482	AK026200	Rn.8291	11.0
Rattus norvegicus cDNA clone UI-R-AF0-yb-o-08-0-UI 3' hypothetical Integrin A (or I) domain structure containing protein	AI711379	N.A.	Rn.40984	10.9
Rattus norvegicus cDNA clone UI-R-C0-hl-d-02-0-UI 3' similar to Necdin-like protein 1 and melanoma antigen, family L, 2 (Magel2), involved in Prader-Willi syndrome	AA996569	THC1265135 THC1265136 THC1250031 THC1289194 THC1294594 THC1331909	0	10.8
Rat TTF-1 mRNA for thyroid nuclear factor 1.	X53953	HSU33749	Rn.34265	10.4
Rattus norvegicus similar to DEME-6 (differentially expressed in MCF7 with estradiol) contains putative RNA binding region RNP-1 signature and Highly hydrophobic region that may represent an anchoring transmembrane domain	XM_216403	BC004399	Mm.23454	9.3
Rattus norvegicus cDNA clone UI-R-CA0-awx-b-10-0-UI 3' found only in f brain library	BE107813	N.A.	Rn.69045	9.2
unnamed protein product (similar to vesicular membrane protein VMP)	BAB15613	AK026982	0	9.2
Rat gene for decay-accelerating factor, partial cds, alternative exons GPI/3'UT and TM/3'UT.	AB032395	M30142	Rn.18841	9.2
Rat mRNA for RT1.A3(O) alpha chain.	X90374	U64801	Rn.39743	9.0
Rat enkephalinase (neutral endopeptidase) mRNA.	M15944	THC1257646 THC1257647	Rn.33598	8.9
Rattus norvegicus cDNA clone UI-R-BO1-ajs-b-09-0-UI 3'. Weakly similar to Immunoglobulin superfamily containing leucine-rich repeat, (slit homolog 1?)	AW527248	N.A.	Rn.16271	8.2
Mouse arginine methyltransferase (Prmt2) mRNA, complete cds.	AF169520	THC1257327	0	8.2
Rat huntingtin associated protein (rHAP1-B) mRNA, complete cds, alternatively spliced form.	U38370	U38372	Rn.37430	8.2
Rattus norvegicus cDNA clone UI-R-FJ0-cpx-m-14-0-UI 3'. Similar to hypothetical protein FLJ12505	CA504848	N.A.	Rn.19205	8.1
10-formyltetrahydrofolate dehydrogenase	AAA70429	NP_036322.2 AAC35000.1	0	8.0
Grb 10	I49199	AAH24285.1	Mm.24640	7.7

Figure 5A

Rattus norvegicus cDNA clone UI-R-FF0-cpe-p-10-0-UI 3' 50 nt homologous to Mus musculus transmembrane inner ear (Tmie), mRNA.	BQ781749	N.A.	Rn.49285	7.5
RIKEN full-length enriched, adult male corpora quadrigemina Mus musculus cDNA clone B230214B19 3', MRNA sequence (similar to GPR 39 (G protein-coupled neurotensin receptor)	BB307345	BC040046.1	Mm.32727	7.4

Figure 5B

LC GENELIST RAT

GENE NAME	GB ACCESSION	HUMAN ORTHOLOGS	UNIGENE	RATIO
Rat dopamine beta-hydroxylase mRNA, complete cds.	L12407	THC1294473	Rn.10912	593.9
Rat tyrosine hydroxylase mRNA, complete cds.	M10244	THC1240552	Rn.11082	202.5
reserpine-sensitive vesicular monoamine transporter	A46374	THC1234153	Mm.91919	176.9
Mouse Ccte gene for chaperonin containing TCP-1 epsilon subunit, complete cds.	AB022158	THC1397904	0	100.6
GTP cyclohydrolase I (EC 3.5.4.16) precursor - rat.	A39080	THC1286828	Rn.2987	69.1
unnamed protein product	BAB14064	AK022504	0	67.4
Rattus norvegicus neuroglobin (Ngb), mRNA.	NM_033359	THC1366005	Rn.64645	65.8
Mouse Eya2 homolog mRNA, partial cds.	U61111	THC1240427	0	54.1
Rat mRNA for glucokinase, alternatively spliced GK2 (EC 2.7.1.1).	X53588	THC1307452	Rn.10447	41.8
Rat mRNA for plasma glutathione peroxidase precursor, complete cds.	D00680	THC1406411 THC1406413	Rn.1491	40.7
KIAA0605 protein protein hypothetical Thrombospondin type I repeat (TSP1) profile/Thrombospondin type I domain containing protein	BAA25531	NM_014694	0	39.2
Rat copper transporter 1 mRNA, complete cds.	AF268030	THC1226350	Rn.2789	39.1
Rat fructose-1,6-bisphosphatase mRNA, complete cds.	M86240	THC1221788 THC1383457	Rn.33703	35.4
Rat peripherin mRNA, complete cds.	AF031876	THC1397670	Rn.11086	33.6
Rat dopamine beta-hydroxylase mRNA, complete cds.	L12407	THC1294473	Rn.10912	31.4
Rat mRNA for sensory neuron synuclein.	X86789	THC1377630	Rn.10421	29.5
Rat collagen type II mRNA, complete cds.	L48440	THC1376376 THC1376377 THC1389266 THC1390757 THC1390758	Rn.10124	28.4
cytochrome b-561 (Cyb561)	NP_031831	NM_001915.2	Rn.14673	28.3
Rat dopa decarboxylase (DDC) mRNA, complete cds.	M27716	THC1346419 THC1346420	Rn.11064	28.1
Mouse partial mRNA for dysferlin (dysf gene).	AJ242954	NP083153 THC1377266 THC1377267	0	25.9
Rattus norvegicus LOC299289 (LOC299269), mRNA weakly similar to interferon, alpha-inducible protein 27-like	XM_238467	NM_032036.1 BC032626.1	Rn.16842	24.9
Mouse RIBP (Ribp) mRNA, complete cds.	AF203343	THC1352911	0	23.7
Rattus norvegicus cDNA clone UI-R-DS0-cje-d-16-0-UI 3'	BM383125	N.A.	Rn.43431	23.3
Rattus norvegicus cDNA clone UI-R-C1-lm-g-11-0-UI 5'.	BF559920	THC1301203	Rn.19423	22.6
Rat (Sprague-Dawley) mRNA for growth hormone-releasing hormone -like peptide.	Z24092	*NM_021091 X00094*	0	21.3
Rat Guanine nucleotide-binding protein G(S), alpha subunit (Adenylate cyclase-stimulating G alpha protein).	AF107845	AF105253	Rn.44217	21.0
collectin 34	BAA81747	NM_003438	0	20.3
Mus musculus transcription factor AP-2 beta, full insert sequence	AK028008	THC1373403	Mm.4795	19.2
unnamed protein product, no homology	BAA91309	NP_060388	0	18.9
Rat DCoH gene. Rattus norvegicus 6-pyruvoyl-tetrahydropterin synthase/dimerizationcofactor of hepatocyte nuclear factor 1 alpha (Pcbd). mRNA.	AJ005542	THC1251737	Rn.7223	17.1
Rattus norvegicus cDNA clone UI-R-A0-bf-d-10-0-UI 3' similar to emb|X69319|HSMAOP14 H.sapiens DNA for monoamine oxidase type A (14)	AA859857	X69319 X60541	Rn.16867	16.9
Mxi 2 protein (Mxi2)	AY195892	N.A.	0	14.5
SKAP55	CAA72101	Y11215	0	13.5
Mus musculus cDNA clone UI-M-DJ2-bwb-j-08-0-UI 3'	BQ174977	N.A.	Mm.153072	13.4
Rattus norvegicus cDNA clone UI-R-EA0-ckw-l-06-0-UI 3' weakly similar to CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	BQ196724	N.A.	Rn.27228	13.0
Rat inhibitor of apoptosis protein 1 mRNA, complete cds.	AF183430	THC1364516	Rn.64578	13.0
Mouse pigment epithelium-derived factor (PEDF) mRNA, complete cds.	AF036164	NP425702 THC1394190	0	12.7
EST218753 Normalized rat muscle, Bento Soares Rattus sp. cDNA clone RMUCE33 3' end.	AI175220	N.A.	Rn.2480	12.4
weakly similar to erythrocyte protein band 4.1-like 4b; expressed in high-metastatic cells				
Mouse pigment epithelium-derived factor (PEDF) mRNA, complete cds.	AF036164	NP425702 THC1394190		12.2
Rat aldehyde dehydrogenase mRNA, complete cds.	J03637	THC1271968 THC1357704 THC1365158	Rn.9810	12.0
Rat monoamine oxidase A gene, complete cds.	D00688	THC1251616	0	11.8
Mouse argininosuccinate synthetase (Ass) mRNA, complete cds.	M31690	THC1396032 THC1396033	0	11.6
Rattus norvegicus cDNA clone UI-R-CX0-bwo-c-06-0-UI 3' similar to cytochrome P450, subfamily IIS, polypeptide 1	BI274639	N.A.	Rn.6389	11.5
Rattus norvegicus cDNA clone UI-R-C2p-qn-b-09-0-UI 3'.45nt similar to hypothetical Eukaryotic thiol (cysteine) proteases active site containing protein	AI555069	N.A.	Rn.17968	11.4
Rat lens mRNA for aldose reductase (AR,EC 1.1.1.21).	X05884	NP115322 THC1406674	Rn.2917	11.3

Figure 6A

Mus musculus adult male olfactory brain cDNA, RIKEN full-length enriched library, clone:6430550H21 product:HYPOTHETICAL 38.5 KDA PROTEIN homolog	AK078264	BC047054	0	11.3
Rat cell growth regulator rCGR11 mRNA, complete cds.	U66470	THC1271663	Rn.31842	11.0
Mouse mRNA for T-cadherin, complete cds.	AB022100	THC1352217 THC1352216	0	11.0
Mouse ectonucleoside triphosphate diphosphohydrolase 3	AK046218	THC1265535		10.9
unnamed protein product contains CUE domain. Domain that may be involved in binding ubiquitin-conjugating enzymes (UBCs). CUE domains also occur in two protein of the IL-1 signal transduction pathway, tollip and TAB2	BAA91357	NM_017949	0	10.9
Rat mRNA for Rab3B protein.	Y14019	THC1359097	Rn.3788	10.8
Rat gamma glutamyl transpeptidase-related enzyme mRNA, partial cds.	U76252	THC1331490	Rn.44367	10.8
Rat mRNA for brain-enriched membrane-associated protein tyrosine phosphatase (BEM)-2, complete cds.	D45413	THC1247043	Rn.10285	10.6
Rattus norvegicus cDNA clone UI-R-BT1-ako-f-11-0-UI 5', Similar to sodium-dependent bile acid transporter	BF565662	N.A.	Rn.46917	10.5
Mouse chromosome 10 popeye protein 3 (Pop3) mRNA, complete cds.	AF204176	NM_022361	0	10.2
Mouse arginine methyltransferase (Prmt2) mRNA, complete cds.	AF169620	THC1257327	0	9.9
Rattus norvegicus cDNA clone UI-R-E0-by-d-10-0-UI 3'.	AA900637	N.A.	Rn.32465	9.7
Rat mRNA for pepsinogen F protein, strain SD.	AJ251687	THC1404692	Rn.43875	9.5
neuronal tetraspanin	AAF19031	NM_130783	0	9.5
Rat clone 53.1.1.1 unknown Glu-Pro dipeptide repeat protein mRNA, complete cds.	U40627	THC1221082	Rn.65864	9.2
Rattus norvegicus cDNA clone UI-R-DM1-ckc-l-19-0-UI 3'.	BM391951	N.A.	Rn.57622	9.1
Rat mRNA for RT1.A3(O) alpha chain.	X90374	U64801	Rn.39743	9.0
Mouse serine protease inhibitor (SPI3) mRNA, complete cds.	U25844	THC1396848	0	9.0
Rattus norvegicus cDNA clone UI-R-FF0-cpe-p-10-0-UI 3' 50 nt homologous to Mus musculus transmembrane inner ear (Tmie), mRNA.	BQ781749	N.A.	Rn.49285	8.8
alpha-fetoprotein precursor	AAA69559	NM_001134	0	8.8
Rat cytokine receptor-like protein CYRL mRNA, complete cds.	AF030243	N.A.	Rn.11944	8.7
Rat gene for decay-accelarating factor, partial cds, alternative exons GPI/3'UT and TM/3'UT.	AB032395	M30142	Rn.18841	8.6
Rattus norvegicus similar to RIKEN cDNA 1700006D24 gene [Mus musculus] (LOC207653), mRNA	XM_213444.1	BC010412	0	8.6
Rattus norvegicus cDNA clone UI-R-CA0-cwt-b-10-0-UI 3' found only in 1 brain library	BE107813	N.A.	Rn.69045	8.5
Mouse Cripl mRNA for cysteine-rich protease inhibitor, complete cds.	AB040537	NM_153370	0	8.5
unnamed protein product	BAA91987	XM_040582	0	8.5
D1 dopamine receptor interacting protein calcyon	AAF34714	NM_015722	0	8.4
Rattus norvegicus cDNA clone UI-R-AF0-yb-c-08-0-UI 3' hypothetical integrin A (or I) domain structure containing protein	AI711379	N.A.	Rn.40964	8.3
Rat monoamine oxidase A gene, complete cds.	D00888	THC1251616	0	8.3
Mouse mRNA for N-glycan alpha 2,8-sialyltransferase.	X92552	THC1307655	0	8.1
Rat mRNA for chloride channels (ClC-K2L and ClC-K2S), complete cds.	D23111	NM_004070	0	8.0

Figure 6B

VTA GENELIST RAT

GENE NAME	GB ACCESSION	HUMAN ORTHOLOGS	UNIGENE	RATIO
Rat tyrosine hydroxylase mRNA, complete cds.	M10244	THC1240552	Rn.11082	311.6
Rattus norvegicus cDNA clone UI-R-EA0-ckx-k-09-0-UI 3' similar to solute carrier family 39 (zinc transporter), member 4 [Homo sapiens] and Catecholamines up protein	BQ196656	THC1277892	Rn.7960	260.2
reserpine-sensitive vesicular monoamine transporter	A46374	THC1234153	Mm.91919	201.9
GTP cyclohydrolase I (EC 3.5.4.16) precursor - rat.	A39080	THC1286628	Rn.2987	61.4
Rat dopa decarboxylase (DDC) mRNA, complete cds.	M27716	THC1346419 THC1346420	Rn.11064	54.9
bA524D16A.1 (sushi-repeat-containing protein)	CAC16060	NM_014467	0	37.7
Rat aldehyde dehydrogenase (ALDH) mRNA, complete cds.	AF001896	THC1394263	Rn.6132	31.7
Rat RET ligand 1 (RETL1) mRNA, complete cds.	U97142	THC1301102	Rn.6281	31.2
Mouse laminin beta3 (Lamb3) chain mRNA, complete cds.	U43298	THC1364202	0	24.7
unnamed protein product	BAA91341	BC047054	0	22.5
Mus musculus adult male olfactory brain cDNA, RIKEN full-length enriched library, clone:6430550H21 product:HYPOTHETICAL 38.5 KDA PROTEIN homolog	AK078264	BC047054	0	20.1
Rat kidney extracellular calcium-sensing receptor mRNA, complete cds.	U10354	THC1259935	Rn.10019	19.9
Mouse lysyl hydroxylase isoform 2 mRNA, complete cds.	AF080572	THC1345653 THC1345656	0	18.1
Rattus norvegicus cDNA clone UI-R-A1-do-b-05-0-UI 3'	AA901088	AL157475	Rn.15183	17.9
dJ888G8.3 (Similar to chelonianin (basic protease inhibitor))	CAC18560	NM_080753	0	17.9
Rat aldehyde dehydrogenase mRNA, complete cds.	M23995	THC1394263	Rn.74044	17.8
Rat mRNA for receptor tyrosine kinase (Ret gene), isoform RET9.	AJ299017	THC1228277 THC1266236 THC1288817 THC1307752 THC1376376 THC1376377	Rn.44178	17.1
Rat collagen type II mRNA, complete cds.	L48440	THC1389266 THC1390757 THC1390758	Rn.10124	16.7
Rat mRNA for keratin 18.	X81448	THC1401834	0	15.7
Mouse DNA for Gpx2 pseudogene.	X91864	THC1345342	0	15.1
Mouse DHFR gene, exon 3.	V00737	XM_114312.3	0	14.3
Rattus norvegicus cDNA clone UI-R-DK0-cez-g-04-0-UI 3' Weakly similar to Notch 3 [Rattus norvegicus]	BI296384	N.A.	Rn.21560	14.0
Mouse mRNA for Vanin-1.	AJ132098	THC1319655	0	12.2
Rat mRNA for RT1.A3(O) alpha chain.	X90374	U64801	Rn.39743	12.2
Rat G-protein coupled thrombin receptor (PAR-1) mRNA, complete cds.	M81642	THC1233563	Rn.2609	11.6
Mouse cyclin ania-6b gene, partial sequence.	AF185591	N.A.	0	11.5
Mouse retinal short-chain dehydrogenase/reductase 1 (Rsdr1) gene, exon 1.	AF179238	BU388706	0	11.3
Rat aldehyde dehydrogenase mRNA, complete cds.	M23995	THC1394263	Rn.74044	11.2
unnamed protein product (similar to vesicular membrane protein VMP)	BAB15613	AK026982	0	11.1
Rat peripherin mRNA, complete cds.	AF031878	THC1397670	Rn.11086	10.9
Mouse unknown protein mRNA, complete cds.	U69172	THC1320139	0	10.6
Rat mRNA for plasma glutathione peroxidase precursor, complete cds.	D00680	THC1406411 THC1406413	Rn.1491	10.5
Rattus norvegicus cDNA clone UI-R-FF0-cps-p-10-0-UI 3' 60 nt homologous to Mus musculus transmembrane inner ear (Tmie), mRNA.	BQ791749	N.A.	Rn.48205	10.5
Mouse fibroblast growth factor 1 gene, exon 3, complete sequence; and partial cds.	AF067190	THC1313049 THC1354397	0	10.1
D1 dopamine receptor interacting protein calcyon	AAF34714	NM_015722	0	10.1
Rattus norvegicus cDNA clone UI-R-E1-fd-f-03-0-UI 5'. Similar to RNA binding motif, single stranded interacting protein 3; RNA-binding protein [Homo sapiens]	BF561068	AK097311	Rn.8943	9.6
Mouse argininosuccinate synthetase (Ass) mRNA, complete cds.	M31690	THC1396032 THC1396033	0	9.5
Rat cell growth regulator rCGR11 mRNA, complete cds.	U66470	THC1271663	Rn.31642	9.3
Wnt2: wingless-type MMTV integration site 2	AK045120	THC1325821	0	9.2
Rat phospholipase C gamma-2 , complete cds.	J05155	THC1233515	Rn.9751	9.1
Rat mRNA for N-G,N-G-dimethylarginine dimethylaminohydrolase, complete cds.	D86041	THC1264320	Rn.4241	9.1
Ca2+ dependent activator protein for secretion; similar to D86214 (NID:g1398903)	AAC14062	NM_017954	0	8.9
Rat follistatin gene, exon 6, clones pROF(301-305).	M31591	THC1240259	0	8.9
Rat gene for decay-accelarating factor, partial cds, alternative exons GPI/3'UT and TM/3'UT.	AB032395	M30142	Rn.18841	8.9
Rat core protein (HSPG) mRNA, complete cds.	M81687	THC1299139	Rn.11127	8.8
Rat glutamate receptor interacting protein 2 mRNA, complete cds.	AF205193	THC1222009	Rn.15680	8.8
Mouse p53 apoptosis-associated target (Perp) mRNA, complete cds.	AF249870	THC1344812 THC1344813	0	8.6
Mouse mRNA for caspase-7.	Y13088	THC1313504	0	8.4

Figure 7A

Rat nicotinic acetylcholine receptor alpha 4 subunit mRNA, complete cds.	L31620	THC1228480	Rn.9697	8.2
cystatin E	AAB81305	NP_001314	0	8.1
Rat DCoH gene. Rattus norvegicus 6-pyruvoyl-tetrahydropterin synthase/dimerizationcofactor of hepatocyte nuclear factor 1 alpha (Pcbd), mRNA.	AJ005542	THC1251737	Rn.7223	8.1
Rattus norvegicus cDNA clone UI-R-DN0-civ-h-20-0-UI 3' similar to Gcnt2 Glucosaminyltransferase, I-branching enzyme	BI292435	N.A.	Rn.6807	8.0

Figure 7B

SN GENELIST RAT

GENE NAME	GB ACCESSION	HUMAN ORTHOLOGS	UNIGENE	RATIO
Rat tyrosine hydroxylase mRNA, complete cds.	M10244	THC1240552	Rn.11082	283.4
reserpine-sensitive vesicular monoamine transporter	A46374	THC1234153	Mm.91919	248.7
Rattus norvegicus cDNA clone UI-R-EA0-clx-k-09-0-UI 3' similar to solute carrier family 39 (zinc transporter), member 4 [Homo sapiens] and Catecholamines up protein	BQ196656	THC1277892	Rn.7960	103.6
Rat dopa decarboxylase (DDC) mRNA, complete cds.	M27716	THC1346419 THC1346420	Rn.11064	77.4
Mouse DNA for Gpx2 pseudogene.	X91884	THC1345342	0	51.0
GTP cyclohydrolase I (EC 3.5.4.16) precursor - rat.	A39080	THC1286628	Rn.2987	35.9
Rat mRNA for sensory neuron synuclein.	X86789	THC1377630	Rn.10421	28.1
Mouse fibroblast growth factor 1 gene, exon 3, complete sequence; and partial cds.	AF067190	THC1313049 THC1354397	0	28.0
Rat RET ligand 1 (RETL1) mRNA, complete cds.	U97142	THC1301102	Rn.6281	27.6
Rat collagen type II mRNA, complete cds.	L48440	THC1376376 THC1376377 THC1389266 THC1390757 THC1390758	Rn.10124	25.1
Rattus norvegicus cDNA clone UI-R-DK0-cez-g-04-0-UI 3' Weakly similar to Notch 3 [Rattus norvegicus]	BI296384	N.A.	Rn.21560	24.8
Mus musculus adult male olfactory brain cDNA, RIKEN full-length enriched library, clone:6430550H21 product:HYPOTHETICAL 38.5 KDA PROTEIN homolog	AK078264	BC047054	0	24.6
Rat aldehyde dehydrogenase (ALDH) mRNA, complete cds.	AF001696	THC1394263	Rn.6132	23.3
Rattus norvegicus cDNA clone UI-R-A1-do-b-05-0-UI 3'	AA901088	AL157475	Rn.15183	19.9
Rat mRNA for receptor tyrosine kinase (Ret gene), isoform RET9.	AJ299017	THC1228277 THC1266236 THC1288817 THC1307752	Rn.44178	18.7
Mouse cyclin ania-6b gene, partial sequence.	AF185591	N.A.	0	18.0
Mouse 4.5S RNA gene.	M12658		0	14.0
Rat mRNA for RT1.A3(O) alpha chain.	X90374	U64801	Rn.39743	13.7
Mouse p53 apoptosis-associated target (Perp) mRNA, complete cds.	AF249870	THC1344812 THC1344813	0	13.1
Rat gene for decay-accelarating factor, partial cds, alternative exons GPI/3'UT and TM/3'UT.	AB032395	M30142	Rn.18841	12.7
Rat mRNA for brain-enriched membrane-associated protein tyrosine phosphatase (BEM)-2, complete cds.	D45413	THC1247043	Rn.10265	12.5
bA524D16A.1 (sushi-repeat-containing protein)	CAC16050	NM_014467	0	12.5
Mouse laminin beta3 (Lamb3) chain mRNA, complete cds.	U43288	THC1364202	0	12.3
Rat periphetin mRNA, complete cds.	AF031878	THC1397870	Rn.11088	12.0
SKAP55	CAA72101	Y11215	0	11.9
Mouse Down syndrome candidate region 1-like protein 1 (Dscr1l1) mRNA, complete cds.	AF237697	THC1228844	0	11.9
Mouse mRNA for caspase-7.	Y13088	THC1313504	0	11.6
Mouse chromosome 17 clone GS-384N12, complete sequence.	AC087217	N.A.	0	11.4
Rat aldehyde dehydrogenase mRNA, complete cds.	M23995	THC1394263	Rn.74044	11.3
Mouse mRNA for Ftp-1, complete cds.	D88187	THC1221341 THC1253645	0	10.7
Rattus norvegicus cDNA clone UI-R-A0-bh-h-03-0-UI 3' similar H.sapiens mRNA for IL13 receptor alpha-1 chain.	AA819359	NM_001560	Rn.2142	10.6
Mouse Dp1l1 mRNA for polyposis locus protein 1-like 1 (TB2 protein-like 1), complete cds.	AB039933	AK058112	0	10.5
Rat mRNA for 2,3-oxidosqualene:lanosterol cyclase, complete cds.	D45252	THC1336224 THC1246851	Rn.10211	10.3
Ca2+ dependent activator protein for secretion; similar to D86214 (NID:g1398903)	AAC14062	NM_017954	0	10.1
Rattus norvegicus cDNA clone UI-R-E1-fd-f-03-0-UI 5'. Similar to RNA binding motif, single stranded interacting protein 3; RNA-binding protein [Homo sapiens]	BF561068	AK097311	Rn.8943	10.0
Rat cell growth regulator rCGR11 mRNA, complete cds.	U66470	THC1271663	Rn.31842	10.0
Rattus norvegicus cDNA clone UI-R-FF0-cpe-p-10-0-UI 3' 50 nt homologous to Mus musculus transmembrane inner ear (Tmie), mRNA.	BQ781749	N.A.	Rn.49285	9.9
Rattus norvegicus cDNA clone UI-R-CY0-bxq-f-10-0-UI 3'.	BI277484	N.A.	Rn.24081	9.9
unnamed protein product	BAA91341	BC047054	0	9.5
Sif3	AAG36772	AF133270.	0	9.4
Rat glutamate receptor interacting protein 2 mRNA, complete cds.	AF205193	THC1222009	Rn.15680	9.3
Rat nicotinic acetylcholine receptor alpha 4 subunit mRNA, complete cds.	L31620	THC1228480	Rn.9697	9.3
Rat mRNA for EGP-314 protein homologue.	AJ001044	THC1232739 THC1219754	Rn.24930	9.2
Mouse NAD(+)-dependent 15-hydroxyprostaglandin dehydrogenase mRNA, complete cds.	U44389	THC1384269	0	8.7
Mouse DHFR gene, exon 3.	V00737	XM_114312.3	0	8.7
Rat thymosin beta-10 gene, complete cds.	M58405	THC1393929	Rn.5983	8.6
Rat thymosin beta-10 gene, complete cds.	M58405	THC1393929	Rn.5983	8.5
Rattus norvegicus cDNA clone UI-R-CN1-cly-a-20-0-UI 3'.	BQ194193	THC1253133	Rn.38662	8.5

Figure 8A

Mouse mRNA for AT motif-binding factor (ATBF1), complete cds.	D26046	THC1271683 THC1288347, THC1288348	0	8.5
Rat G-protein coupled thrombin receptor (PAR-1) mRNA, complete cds.	M81642	THC1233563	Rn.2609	8.4
Rattus norvegicus cDNA clone UI-R-DK0-ce-b-12-0-UI 3'.	BI294080	N.A.	Rn.1135	8.3
Rattus norvegicus cDNA clone UI-R-C2p-oc-a-05-0-UI 5'.	BF551935	N.A.	Rn.1135	8.2
Rattus norvegicus glycoprotein hormone alpha 2 (Gpha2), mRNA	NM_133619.2	NM_130769	Rn.22078	8.0

Figure 8B

SN>VTA GENELIST RAT

GENE NAME	GB ACCESSION	HUMAN ORTHOLOGS	UNIGENE	RATIO SN/VTA
Rat mRNA for sensory neuron synuclein.	X86789	THC1377630	Rn.10421	5.5
Rat protein kinase C delta subspecies.	M16330	THC1264191	0	5.1
DRNBTE01 Rat DRG Library Rattus norvegicus cDNA clone DRNBTE01 5'.	BG673363	N.A.	0	4.6
Rat Sprague-Dawley N-methyl-D-aspartate receptor NMDAR2C subunit mRNA, complete cds.	U08259	THC1384683 THC1384684	Rn.9709	4.6
retinoic acid-inducible gene 1 (RAIG1): putative G protein-coupled receptor	BC039217	BC003665	0	4.4
Sox6 (SRY-box containing gene 6)	AK044981	AK021785.	Mm.4656	4.0
Rattus norvegicus similar to RPE-spondin [Homo sapiens] (LOC297757), mRNA	XM_216317	AY040546	0	4.0
Rat heat shock protein 22 mRNA, complete cds.	AF314540	THC1351514	Rn.48843	3.8
Rat osteoactivin mRNA, complete cds.	AF184983	THC1277545	Rn.13778	3.8
Sox6 (SRY-box containing gene 6)	AK044981	AK021785.	Mm.4656	3.6
Mouse thrombospondin (THBS1) gene, exon 22 and complete cds.	M62470	THC1404645	0	3.5
Rat testis-specific histone H1t and histone H4t, complete cds.	M28409	THC1234494 THC1366165 THC1379006 THC1385076 THC1394032	Rn.48806	3.4
Rattus norvegicus cDNA clone UI-R-BT0-pr-e-01-0-UI 3'.	AI144777	AK055409	Rn.8715	3.0

Figure 9

VTA>SN GENELIST RAT

GENE NAME	GB ACCESSION	HUMAN ORTHOLOGS	UNIGENE	RATIO SN/VTA
Mouse unknown protein mRNA, complete cds.	U69172	THC1320139	0	-27.6
Rat CDK108 mRNA.	Y17328	THC1384210	Rn.24561	-27.4
Rat pituitary adenylate cyclase activating polypeptide precursor protein mRNA, complete cds.	M83006	THC1365746	Rn.37400	-17.4
Rat lipoprotein lipase mRNA, complete cds.	L03294	THC1286881	Rn.3834	-17.4
Rat follistatin gene, exon 6, clones pROF(301-305).	M31591	THC1240259	0	-14.1
Mouse mRNA for ertactin.	X14194	THC1363922	0	-11.9
Rat kidney extracellular calcium-sensing receptor mRNA, complete cds.	U10354	THC1259935	Rn.10019	-11.8
Mouse mRNA for K-glypican.	X83577	THC1246446	0	-11.4
CG12746 gene product [alt 1]	AAF52007	XP_219376	0	-9.1
Rattus norvegicus cDNA clone UI-R-C0-jq-d-07-0-UI 3'.	AI045808	THC1360452	Rn.44028	-8.3
Rattus norvegicus cDNA clone UI-R-DR0-cjc-g-03-0-UI 3'.	BI303966	THC1300778	Rn.49567	-8.3
Rat phospholipase C gamma-2 , complete cds.	J05155	THC1233515	Rn.9751	-7.8
Rattus norvegicus G-substrate phosphatase inhibitor mRNA, complete cds.	AF294688	BC028094	Rn.24323	-7.0
Mouse manic fringe precursor mRNA, complete cds.	U94349	THC1324685	0	-6.6
Rat heat stable antigen CD24 mRNA, complete cds.	U49062	THC1391062	Rn.6007	-6.5
Rattus norvegicus chloride intracellular channel 5 (Clic5), mRNA. NM_053603	NM_053603	THC1358746	Rn.1838	-6.5
Rat synaptotagmin interacting protein STIP2 mRNA, complete cds.	AF193757	THC1221973	Rn.15796	-6.0
hypothetical protein	CAB94883	AB04679	0	-5.9
Mouse mRNA for collagen type XIV, partial.	AJ131395	BC014640	0	-5.7
Rat differentation-associated Na-dependent inorganic phosphate cotransporter (DNPI) mRNA, complete cds.	AF271235	THC1253617	Rn.19372	-5.4
Rattus norvegicus cDNA clone UI-R-C0-gs-c-02-0-UI 3'.	AA963971	N.A.	Rn.7936	-5.4
Rat PND gene encoding atrial natriuretic factor, complete cds.	K02062	THC1305948	Rn.2004	-5.1
Rattus norvegicus cDNA clone UI-R-CV0-brm-d-06-0-UI 3'.	BG372104	N.A.	Rn.22267	-5.1
Rat neuropilin mRNA, complete cds.	AF016296	THC1311825	Rn.10815	-5.0
Mouse mRNA for Doc2beta, complete cds.	D85037	THC1235104	0	-4.9
KIAA1613 protein	BAB13439	AB046833	0	-4.5
Rat E11 antigen epitope (OTS-8) mRNA, complete cds.	U32115	THC1376086	Rn.794	-4.5
Rat mRNA for collagen alpha 2 type V, partial cds.	AJ224880	THC1389266 THC1389270	Rn.2875	-4.4
Rat mRNA for Rab3B protein.	Y14019	THC1359097	Rn.3788	-4.4
Rattus norvegicus cDNA clone UI-R-DK0-cea-c-05-0-UI 3'.	BI294233	THC1265487	Rn.43469	-4.3
tuftelin	AAC04577	NM_020127	0	-4.3
Rat mRNA for Castration Induced Prostatic Apoptosis Related protein-1 (CIPAR-1).	AJ010750	THC1239865	Rn.21667	-4.2
Rattus norvegicus cDNA clone UI-R-BU0-apb-e-03-0-UI 3'.	BE095865	AF393329	Rn.21852	-4.2
Rat GlcAT-S mRNA for UDP-glucuronyltransferase-S, complete cds.	AB010441	THC1294198	Rn.42869	-4.2
Mouse lysyl hydroxylase isoform 2 mRNA, complete cds.	AF080572	THC1345653 THC1345656	0	-4.1
Rat DRG Library Rattus norvegicus cDNA clone DRNBLF02 5'.	BG671101	AK026200	Rn.3291	-4.1
Mouse Ephrin B3	AK046305.1	BC042844	0	-4.1
Rattus norvegicus cDNA clone UI-R-C4-ziq-h-05-0-UI 3'.	AW534235	N.A.	Rn.68154	-3.9
Rattus norvegicus cDNA clone UI-R-BU0-apl-f-06-0-UI 3'.	BE099688	N.A.	Rn.46962	-3.9
Rat fibrillin-1 mRNA, complete cds.	AF135059	THC1251322	Rn.12759	-3.9
Rat vanilloid receptor splice variant mRNA, complete cds.	AF158248	AY131289	Rn.3073	-3.9
Mouse mRNA for adhesion protein RA175N, complete cds.	AB021967	BC047021.1	0	-3.8
Rat neuronal olfactomedin-related ER localized protein (D2Sut1e) mRNA, complete cds.	U03417	THC1382619	Rn.11005	-3.7
Rat insulin-like growth factor binding protein 5 mRNA, 3' UTR.	AF139830	BC011453	Rn.1593	-3.7
Mouse C1q-related factor mRNA, complete cds.	AF095155	THC1346519	0	-3.6
Rat RND gene encoding atrial natriuretic factor, complete cds.	K02062	THC1305948	Rn.2004	-3.6
Wnt2: wingless-type MMTV integration site 2	AK045120.1	NM_003391.1	0	-3.5
Rat nerve growth factor-induced (NGFI-A) gene, complete cds.	M18416	THC1299115	Rn.9096	-3.5
Immunoglobulin superfamily, member 4, IGSF4	AF434663.1	AF132811	Mm.20921	-3.4
Murine genomic DNA; partially digested Sau3A fragment, cloned into cosmid vector pEMBLcos2, complete sequence.	AF059580	N.A.	0	-3.4
Immunoglobulin superfamily, member 4, IGSF4	AF434663.1	AF132811	Mm.20921	-3.4
Rattus norvegicus cDNA clone UI-R-BT1-akv-g-05-0-UI 3'.	AW531194	THC1222472	Rn.46621	-3.2
axonemal dynein heavy chain	CAB94756	BAB13405.1	0	-3.2
unnamed protein product	BAA91922	NM_018281	0	-3.2
Rat fibrillin-1 mRNA, complete cds.	AF135059	THC1251322	Rn.12759	-3.2
Rat purine-selective sodium/nucleoside cotransporter (rCNT2) mRNA, complete cds.	U66723	THC1332607	Rn.10140	-3.2
extensin-like protein	CAA84230	N.A.	0	-3.1
Mouse chordin mRNA, complete cds.	AF096276	THC1223192 THC1342514	0	-3.1
axonemal dynein heavy chain	CAB94756	BAB13405.1	0	-3.0
Rattus norvegicus cDNA clone UI-R-CN1-cjm-g-13-0-UI 3'.	BM390981	N.A.	Rn.23137	-3.0

Figure 10

SN GENELIST HUMAN

GENE NAME	GB ACCESSION	RATIO
tyrosine hydroxylase	M17589	65.0
dopa decarboxylase (aromatic L-amino acid decarboxylase)	M76180	58.4
tyrosine hydroxylase	L15440	53.6
solute carrier family 6 (neurotransmitter transporter, dopamine), member 3	D88570	50.0
aldehyde dehydrogenase 1, soluble	K03000	22.5
glutathione peroxidase 3 (plasma)	D16362	19.0
dopamine receptor D2	X51362	17.4
ATPase, Ca++ transporting, ubiquitous	NM_005173	16.5
hepatocyte nuclear factor 3, alpha	U39840	15.6
KIAA1051 protein	AF038187	15.3
dopamine receptor D2	S62137	14.1
myogenin (myogenic factor 4)	X17651	14.0
killer cell lectin-like receptor subfamily C, member 4	AF027164	14.0
dystrophia myotonica-containing WD repeat motif	L19267	13.9
N-acetyltransferase 1 (arylamine N-acetyltransferase)	AF071552	13.9
similar to Xenopus laevis gamma-crystallin 6; similar to AF071563 (PID:g3930581)	AAD15549	13.8
Tubulin, alpha, brain-specific	AF141347	13.6
small inducible cytokine subfamily A (Cys-Cys), member 13	U46767	13.6
small inducible cytokine subfamily A (Cys-Cys), member 13	U59808	13.6
Human pepsinogen gene, exon 9, clone PCG401.	M23077	13.3
TYRO protein tyrosine kinase binding protein	AF019562	13.2
interleukin 17 (cytotoxic T-lymphocyte-associated serine esterase 8)	U32659	13.2
microseminoprotein, beta-	S67815	13.2
paired box gene 9	U59628	12.9
ficolin (collagen/fibrinogen domain-containing lectin) 2 (hucolin)	D49353	12.7
sodium-dependent bile acid transporter	BAA19846	12.5
Sequence 82 from Patent WO9953040.	AX014884	12.4
olfactory receptor, family 10, subfamily J, member 1	X64995	12.1
homeo box C4	X07495	11.9
E3 ubiquitin ligase SMURF1	AB046845	11.9
bromodomain, testis-specific	AA884041	11.8
2_19	AAA92652	11.6
cynuclein, gamma (breast cancer-specific protein 1)	AA804675	11.6
H3 histone family, member K	NM_003526	11.5
myogenin (myogenic factor 4)	X17651	11.5
Human U5E snRNA sequence.	M77839	11.4
membrane-spanning 4-domains, subfamily A, member 1	M89796	11.3
TLR9	BAA76331	11.2
RAB10, member RAS oncogene family	AK001555	11.2
cartilage intermediate layer protein, nucleotide pyrophosphohydrolase	NM_003613	11.1
neurofilament, heavy polypeptide (200kD)	X15306	11.0
Human c-jun gene, promoter region with flanking evolutionary conserved sequences.	U60581	10.9
Homo sapiens chromosome 14q24.3 clone BAC270M14 transforming growth factor-beta 3 (TGF-beta 3) gene, complete cds; and unknown genes	AF107095	10.9
Homo sapiens cDNA FLJ11979 fis, clone HEMBB1001282, weakly similar to ANKYRIN R	AK022041	10.9
solute carrier family 3 (cystine, dibasic and neutral amino acid transporters, activator of cystine, dibasic and neutral amino acid transport), member 1	M95548	10.9
small inducible cytokine A1 (I-309, homologous to mouse Tca-3)	M57502	10.7
collagen, type IV, alpha 6	D21337	10.7
Human CIG30 gene, partial cds.	AF292387	10.7
Human CpG island DNA genomic Mse1 fragment, clone 29e4, forward read cpg29e4.ft1a.	Z60599	10.6
protein kinase, cAMP-dependent, regulatory, type II, alpha	X14968	10.5
CD2 antigen (p50), sheep red blood cell receptor	M16336	10.5
L2DTL protein	AF195765	10.4
DKFZP586N1922 protein	AL117468	10.2
ATP/ADP translocator	AAA33027	10.2
transient receptor potential-related channel 7, a novel putative Ca2+ channel protein	BAA95563	10.2
keratin 6	AI978932	10.2
breast cancer 1, early onset	L78833	10.2
solute carrier family 16 (monocarboxylic acid transporters), member 6	AK000416	10.1
Human DiGeorge syndrome critical region, centromeric end.	L77570	10.0

Figure 11A

SMS3 protein	AB029488	10.0
matrix metalloproteinase-like 1	AJ003147	10.0
Human hXBP-1 transcription factor DNA.	L13850	10.0
Human genomic DNA, chromosome 21q, section 36/105.	AP001692	9.9
hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	AF207601	9.8
hypothetical protein FLJ10441	AK023155	9.8
aldolase A, fructose-bisphosphate	BE252246	9.8
cadherin 6, type 2, K-cadherin (fetal kidney)	D31784	9.8
Human genomic DNA, chromosome 1q22-q23, CD1 region, section 3/4.	AP002534	9.7
Human c-Ha-ras2 oncogene (Harvey ras family).	X00419	9.7
putative translation initiation factor	AF083441	9.7
interleukin 1 receptor-like 1	AB012701	9.6
survival of motor neuron 1, telomeric	U80017	9.6
Homo sapiens clone 24566 mRNA sequence	AF070536	9.6
Sequence 20 from Patent WO9947669.	AX017269	9.5
Homo sapiens cDNA FLJ13236 fis, clone OVARC1000408	AK023298	9.5
bromodomain adjacent to zinc finger domain, 1B	AF084479	9.5
interleukin 13	NM_002188	9.5
chemokine (C-C motif) receptor 2	U03882	9.4
similar to ankyrin of Chromatium vinosum.	BAA11489	9.4
G protein-coupled receptor 38	AF034632	9.4
solute carrier family 26, member 3	L02785	9.3
phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	M14636	9.3
spectrin, alpha, erythrocytic 1 (elliptocytosis 2)	AA703344	9.3
budding uninhibited by benzimidazoles 1 (yeast homolog)	AF053305	9.3
cadherin 6, type 2, K-cadherin (fetal kidney)	D31784	9.2
carcinoembryonic antigen-related cell adhesion molecule 8	X52378	9.2
Human cDNA: FLJ22913 fis, clone KAT06142.	AK026566	9.2
transcription factor AP-2 gamma (activating enhancer-binding protein 2 gamma)	AA632055	9.2
Tubulin, alpha, brain-specific	K00557	9.2
testosterone 16-alpha-hydroxylase	AAA40425	9.2
KIAA0324 protein	AW016078	9.2
KIAA0842 protein	AF243495	9.2
inducible membrane protein	CAA37804	9.1
Homo sapiens cDNA FLJ11041 fis, clone PLACE1004405	AK001803	9.1
Human leucine-rich repeat-containing G-protein-coupled receptor 6 (LGR6) mRNA, partial cds.	AF160501	9.1
Sequence 228 from Patent WO9951727.	AX015935	9.1
membrane protein of cholinergic synaptic vesicles	NM_003373	9.1
hypothetical protein from EUROIMAGE 2260343	AL260143	9.1
matrix metalloproteinase 3 (stromelysin 1, progelatinase)	U78045	9.1
Human ubiquitin carboxy-terminal hydrolase L1 (UCHL1) gene, exon 3.	AF076269	9.0
Homo sapiens cDNA FLJ13070 fis, clone NT2RP3001777	AK024508	9.0
KIAA0042 gene product	D26351	9.0
Kallmann syndrome 1 sequence	AA418499	9.0
hypothetical protein FLJ10748	AK001610	9.0
testis nuclear RNA binding protein	CAA59169	9.0
Sequence 17 from Patent WO9954460.	AX013083	8.9
matrix metalloproteinase-like 1	AJ003147	8.8
ATPase, H+ transporting, lysosomal (vacuolar proton pump) 21kD	BE315015	8.8
Human PAX3/forkhead transcription factor gene fusion mRNA, complete cds.	U02368	8.8
Sjogren syndrome antigen A1 (52kD, ribonucleoprotein autoantigen SS-A/Ro)	M62800	8.7
properdin P factor, complement	X57748	8.7
hypothetical protein	CAB70626	8.7
heterogeneous nuclear ribonucleoprotein H2 (H')	U78027	8.7
Sequence 8 from Patent WO9955858.	AX011612	8.7
SGC32445 protein	AF070551	8.6
Human proline isomerase FK506-binding protein (FKBP13) gene, exons 1-6.	L18980	8.6
Human CpG island DNA genomic Mse1 fragment, clone 59a6, forward read cpg59a6.ft1a.	Z55705	8.6
hyaluronan-binding protein 2	D49742	8.6
ELK4, ETS-domain protein (SRF accessory protein 1)	M85164	8.6
ring finger protein 15	U91328	8.5
homeo box A9	U81511	8.5
chitinase 1 (chitotriosidase)	AI097512	8.5
parathyroid hormone	V00597	8.5
hypothetical protein FLJ22127	AK025539	8.5
breast cell glutaminase	AF038170	8.5

Figure 11B

T-box 2	AK001031	8.5
ring finger protein 15	U91328	8.5
G protein pathway suppressor 1	BE267975	8.5
collagen, type VIII, alpha 2	M60632	8.5
hippocalcin	D16593	8.4
DNA cytosine-5 methyltransferase 3A	AAC40177	8.3
keratin 8	X74929	8.3
alpha,-antitrypsin-like protein	BAA24419	8.3
ankyrin repeat-containing protein	X69838	8.3
mammaglobin 2	AF071219	8.3
SMC (mouse) homolog, X chromosome	L25270	8.2
Homo sapiens mRNA; cDNA DKFZp434P0810 (from clone DKFZp434P0810)	AL137595	8.2
potassium channel subunit	AAC83350	8.2
prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	L15326	8.1
nicotinamide nucleotide transhydrogenase	U40490	8.1
gpStaf50	CAA57684	8.1
Sequence 538 from Patent WO9954461.	AX013595	8.0
smoothelin-C	AAF81786	8.0

Figure 11C

LC GENELIST HUMAN

GENE NAME	GB ACCESSION	RATIO
dopamine beta-hydroxylase (dopamine beta-monooxygenase)	Y00096	100.0
tyrosine hydroxylase	M17589	36.2
tyrosine hydroxylase	L15440	31.7
heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A)	AF068846	15.1
inositol polyphosphate phosphatase-like 1	Y14385	13.5
Human gene for substance P receptor (exon 5).	X65181	11.7
Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	U65092	11.3
activin A receptor, type IIB	AB008681	11.2
transient receptor potential-related channel 7, a novel putative Ca2+ channel protein	BAA95563	11.0
killer cell lectin-like receptor subfamily C, member 4	AF027164	10.6
tachykinin receptor 1	X65177	10.6
N-acetyltransferase 1 (arylamine N-acetyltransferase)	AF071552	10.3
Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1	U65092	10.3
paired box gene 9	U59628	10.2
chemokine (C-C motif) receptor 2	U03882	10.2
Homo sapiens cDNA FLJ11979 fis, clone HEMBB1001282, weakly similar to ANKYRIN R	AK022041	9.5
small inducible cytokine subfamily A (Cys-Cys), member 13	U59808	9.3
zinc finger protein 137 (clone pHZ-30)	U09414	9.3
TYRO protein tyrosine kinase binding protein	AF019562	9.3
Human pepsinogen gene, exon 9, clone PCG401.	M23077	9.2
Human genomic DNA, chromosome 21q, section 36/105.	AP001692	9.2
phosphoribosyl pyrophosphate amidotransferase	D13757	9.1
inducible membrane protein	CAA37804	8.9
Human CpG island DNA genomic Mse1 fragment, clone 29e4, forward read cpg29e4.ft1a.	Z60599	8.8
Homo sapiens cDNA: FLJ20882 fis, clone ADKA03206	AK024535	8.8
ring finger protein 15	U91328	8.7
dopa decarboxylase (aromatic L-amino acid decarboxylase)	M76180	8.7
homeo box C4	X07495	8.6
TLR6	BAA78631	8.4
dystrophia myotonica-containing WD repeat motif	L19267	8.4
E3 ubiquitin ligase SMURF1	AB046845	8.4
ficolin (collagen/fibrinogen domain-containing lectin) 2 (hucolin)	D49353	8.4
myogenin (myogenic factor 4)	X17651	8.4
bromodomain, testis-specific	AA884041	8.2
bromodomain adjacent to zinc finger domain, 1B	AF084479	8.2

Figure 12

Figure 13A

Gene name	GB Acc.	Human Orthologs	Unigene	RATIO SN	RATIO VTA	RATIO A13	RATIO LC
Rat tyrosine hydroxylase mRNA, complete cds.	M10244	NM_199292	Rn.11082	283.5	276.8	149.7	202.5
reserpine-sensitive vesicular monoamine transporter	A46374	N.A.	Mm.91919	248.7	209.8	67.6	176.9
Rat dopa decarboxylase (DDC) mRNA, complete cds.	M27716	NM_000790	Rn.11064	77.4	69.1	40.7	28.1
GTP cyclohydrolase I (EC 3.5.4.16) precursor - rat.	A39080	N.A.	Rn.2987	35.9	71.1	27.0	69.1
Rat collagen type II mRNA, complete cds.	L48440	NM_001844	Rn.10124	25.1	13.3	11.0	28.4
Rat peripherin mRNA, complete cds.	AF031878	N.A.	Rn.11086	12.0	10.1	16.8	33.5
Mus musculus adult male olfactory brain cDNA, RIKEN full-length enriched library, clone:6430550H21 product:HYPOTHETICAL 38.5 KDA PROTEIN homolog	AK078264	BC047054	N.A.	24.5	29.3	6.5	11.3
Rat mRNA for plasma glutathione peroxidase precursor, complete cds.	D00680	NM_002084	Rn.1491	5.2	7.4	16.3	40.7
Rat DCoH gene. Rattus norvegicus 6-pyruvoyl-tetrahydropterin synthase/dimerizationcofactor of hepatocyte nuclear factor 1 alpha (Pcbd), mRNA.	AJ005542	N.A.	Rn.7223	7.0	9.7	13.7	17.1
Rat gene for decay-accelarating factor, partial cds, alternative exons GPI/3'UT and TM/3'UT.	AB032395	N.A.	Rn.18841	12.7	14.0	9.2	8.6
Rat mRNA for RT1.A3(O) alpha chain.	X90374	N.A.	Rn.39743	13.7	11.8	9.0	9.0
Mouse argininosuccinate synthetase (Ass) mRNA, complete cds.	M31690	N.A.	N.A.	6.0	8.6	11.1	11.6
Rattus norvegicus cDNA clone UI-R-FF0-cpe-p-10-0-UI 3' 50 nt homologous to Mus musculus transmembrane inner ear (Tmie), mRNA.	BQ781749	N.A.	Rn.49285	9.9	9.5	7.5	8.8
Mouse mRNA for N-glycan alpha 2,8-sialyltransferase.	X83562	NM_006011	N.A.	4.7	7.9	20.3	8.1
Mouse 4.5S RNA gene.	M12658	N.A.	N.A.	14.0	7.5	6.9	6.0
Mouse arginine methyltransferase (Prmt2) mRNA, complete cds.	AF169620	NM_001535	N.A.	6.8	7.8	8.2	9.9

Figure 13B

unnamed protein product (similar to vesicular membrane protein VMP)	BAB15613	N.A.	N.A.	7.4	9.2	9.2	6.9	
Rat cell growth regulator rCGR11 mRNA, complete cds.	U66470	N.A.	Rn.31842	10.0	7.5	5.2	11.0	
D1 dopamine receptor interacting protein calcyon	AAF34714	N.A.	N.A.	6.4	8.4	7.4	8.4	
Rattus norvegicus similar to DEME-6 (differentially expressed in MCF7 with estradiol) contains putative RNA binding region RNP-1 signature and Highly hydrophobic region that may represent an anchoring transmembrane domain	XM_216483	N.A.	Mm.23454	7.0	7.2	9.6	5.8	
EST218753 similar to erythrocyte protein band 4.1-like 4b; expressed in high-metastatic cells	AI175220	N.A.	Rn.2480	4.6	8.1	5.6	12.4	
Rat mRNA for 2,3-oxidosqualene:lanosterol cyclase, complete cds.	D45252	NM_002340	Rn.10211	10.3	7.3	4.2	7.8	
Rat thymosin beta-10 (testis-specific) gene, complete cds.	M58404	NM_021103	Rn.5983	6.9	8.4	5.4	7.7	
Rattus norvegicus cDNA clone UI-R-CA0-awx-b-10-0-UI 3' found only in 1 brain library	BE107813	N.A.	Rn.69045	6.3	4.3	9.2	8.5	
Rat thymosin beta-10 gene, complete cds.	M58405	NM_021103	Rn.5983	8.6	6.3	4.5	7.7	
Rat thymosin beta-10 gene, complete cds.	M58405	NM_021103	Rn.5983	8.5	6.1	4.4	7.8	
KIAA1402 protein	BAA92640	N.A.	N.A.	7.6	5.4	4.8	7.5	
Apoptosis repressor with CARD domain	U40627	NM_003946	Rn.86956	5.8	5.7	4.7	9.2	
Mouse argininosuccinate synthetase (Ass) mRNA, complete cds.	M31690	N.A.	N.A.	4.1	6.2	7.1	7.9	
CTCL tumor antigen se20-4	AAG34906	N.A.	N.A.	4.8	6.5	6.7	6.8	
intracellular chloride channel CLIC3	AAD16450	N.A.	N.A.	5.3	7.2	5.9	5.2	
Mouse 8-oxodGTPase protein (Mth1) gene, upstream promoter region.	AF291054	N.A.	N.A.	6.1	5.3	5.4	6.3	
Rat PFK-L mRNA for liver phosphofructokinase.	X58865	NM_002626	Rn.16607	7.2	4.7	4.7	7.0	
unnamed protein product	BAB14872	N.A.	N.A.	6.5	5.5	5.9	5.1	
unnamed protein product	BAB15550	N.A.	N.A.	6.1	7.0	4.4	5.3	
Rat 150 kDa oxygen regulated protein (ORP150) mRNA, complete cds.	U41853	N.A.	Rn.10542	5.0	5.9	5.8	5.1	

Figure 13C

conserved hypothetical protein	AAG03939	N.A.	N.A.	5.1	5.7	5.2	5.2
Mouse protein phosphatase X (Ppx) mRNA, complete cds.	AF088911	NM_002720	N.A.	5.2	4.5	4.7	6.6
Mouse mRNA for 3'-end of NCAM-140 and NCAM-180 isoforms.	X15052	NM_000615	N.A.	4.6	5.2	5.7	5.0
Mouse arginine methyltransferase (Prmt2) mRNA, complete cds.	AF169620	NM_001535	N.A.	4.6	4.6	4.8	5.8

Figure 14A

Gene name	GB Acc.	Human Orthologs	Unigene	RATIO SN	RATIO VTA	RATIO A13	RATIO LC
Mouse bone morphogenetic protein 2 (BMP-2) gene, complete cds.	L25602	N.A.	N.A.	1.2	3.8	0.2	1.5
Wnt2: wingless-type MMTV integration site 2	AK045120	NM_003391	N.A.	2.5	9.3	0.1	1.6
Mouse mRNA for distal intestinal serine protease (DISP gene).	AJ243866	N.A.	N.A.	1.4	4.4	0.8	2.0
Rattus norvegicus cDNA clone UI-R-CX0-bwx-e-02-0-UI 3'	BI278837	N.A.	Rn.18159	5.3	2.4	3.5	0.9
Rat RET ligand 1 (RETL1) mRNA, complete cds.	U97142	NM_005264	Rn.6281	27.6	29.9	34.0	2.2
Rattus norvegicus cDNA clone UI-R-A0-bc-f-03-0-UI 3'.	AA818585	N.A.	Rn.16727	1.3	2.8	4.3	0.5
Rat mRNA for very-long-chain acyl-CoA synthetase, complete cds.	D85100	NM_003645	Rn.3608	1.7	3.3	4.7	0.4
Rattus norvegicus putative fatty acid desaturase (Fads3),	XM_215140	N.A.	N.A.	4.5	4.7	6.1	0.5
RIKEN full-length enriched, adult male corpora quadrigemina Mus musculus cDNA clone B230214B19 3', MRNA sequence (similar to GPR 39 (G protein-coupled neurotensin receptor)	BB307345	N.A.	Mm.32727	3.6	4.5	7.4	0.5
SODIUM CHANNEL, VOLTAGE-GATED, TYPE 6, ALPHA (SCN6A)	Y09164	N.A.	Rn.54541	2.6	3.5	3.3	0.7
Rat mRNA for metastasis-associated GPI-anchored protein.	AJ001043	NM_014400	Rn.38434	1.1	2.8	4.2	0.7
Rattus norvegicus cDNA clone UI-R-CA1-bjt-g-12-0-UI 3'.	BF410127	N.A.	N.A.	1.3	2.4	5.5	0.8
Rat mRNA for Castration Induced Prostatic Apoptosis Related protein-1 (CIPAR-1).	AJ010750	N.A.	Rn.21667	1.0	5.0	7.4	1.2
Rat DRG Library Rattus norvegicus cDNA clone DRNBLF02 5'.	BG671101	N.A.	Rn.3291	1.2	5.3	11.0	1.8
Rattus norvegicus cDNA clone UI-R-FJ0-cpx-m-14-0-UI 3'.	CA504848	N.A.	Rn.19205	2.2	5.3	8.1	1.9
Similar to hypothetical protein FLJ12505	AF195419	XM_047995	N.A.	1.6	2.1	5.5	1.1
Mouse ODZ2 (Odz2) mRNA, partial cds.							
Rattus norvegicus cDNA clone UI-R-CX0-bxm-h-07-0-UI 3'							
moderately similar to Tnf receptor-associated factor 3 (Traf3) gene, partial sequence; and amnionless precursor protein (Amn) gene	BI276908	N.A.	Rn.7787	4.4	4.5	25.2	0.8
vitelliform macular dystrophy protein	AAC64926	N.A.	N.A.	2.2	2.2	3.8	0.4
Mouse mimp3 mRNA for igf2 mRNA-binding protein 3, complete cds.	AB046173	N.A.	N.A.	3.0	4.4	1.5	0.6

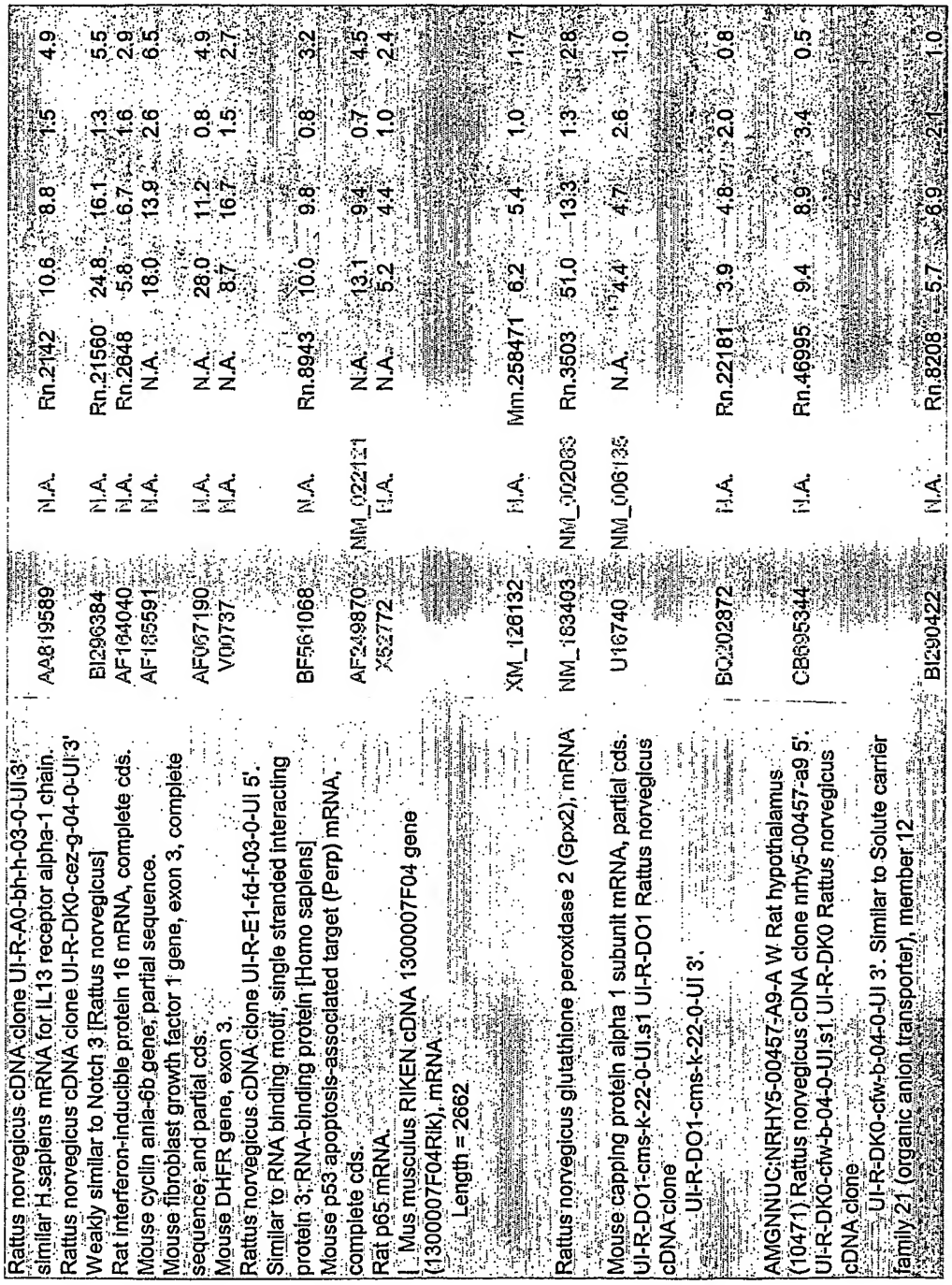
Figure 14B

ESTs Highly similar to insulin-like growth factor 2, binding protein 3	CA503752	N.A.	Rn.31564	2.8	4.0	1.4	0.5	
Mouse untranslated RNA G90.	AJ132433	N.A.	N.A.	5.3	7.3	2.5	0.2	
Rat mRNA for keratin 18.	X81448	N.A.	N.A.	7.1	14.9	3.9	0.1	
Rat nicotinic acetylcholine receptor alpha 4 subunit mRNA, complete cds.	L31620	NM_000744	Rn.9697	9.3	9.6	2.8	0.3	
tyrosine phosphatase receptor Ftp-1	D88187	N.A.	N.A.	10.7	8.2	3.6	0.2	
Rat gene for glycogen phosphorylase (liver type).	X63515	NM_002863	Rn.21399	2.5	4.8	1.7	0.5	
Rat protein kinase C-binding protein NELL1 mRNA, complete cds.	U48246	NM_006157	Rn.10695	3.5	4.5	1.8	0.1	
Rat protein kinase C-binding protein NELL1 mRNA, complete cds.	U48246	NM_006157	Rn.10695	3.2	4.4	1.9	0.1	
Rattus norvegicus LOC302889 (LOC302889), mRNA	XM_239146	N.A.	N.A.	1.7	2.5	2.2	0.2	
Mus musculus similar to phosphoinositol 3-phosphate-binding protein-3 [Homo sapiens] (LOC233765), mRNA	XM_133864	N.A.	N.A.	2.4	2.8	3.5	0.1	
Mus musculus similar to phosphoinositol 3-phosphate-binding protein-3 [Homo sapiens] (LOC233765), mRNA	XM_133864	N.A.	N.A.	2.4	2.8	3.6	0.1	
SN/VTA DA CLUSTER 1								
Rattus norvegicus cDNA clone UI-R-C0-gx-g-08-0-UI 3'.	AA964745	N.A.	Rn.11902	6.6	2.5	0.9	0.8	
Rattus norvegicus cDNA clone UI-R-BJ0p-afu-e-03-0-UI 3'.	AW435438	N.A.	Rn.54366	4.3	2.9	0.9	0.7	
Rat serine protease RNK-Met-1 mRNA, complete cds .	L05175	NM_005317	Rn.9838	1.8	4.7	0.8	0.6	
Mouse PDZ domain actin binding protein Shroom mRNA, complete cds.	AF199421	N.A.	N.A.	2.6	4.4	0.4	0.9	
Mouse zfh-4 mRNA for zinc-finger homeodomain protein 4, complete cds.	AB024499	N.A.	N.A.	4.3	4.9	0.4	0.9	
Rat aldehyde dehydrogenase mRNA, complete cds.	M23995	N.A.	Rn.74044	6.4	16.7	0.7	1.0	
Rat aldehyde dehydrogenase mRNA, complete cds.	M23995	N.A.	Rn.74044	11.3	22.5	0.4	0.9	
Rat aldehyde dehydrogenase (ALDH) mRNA, complete cds.	AF001896	NM_000689	Rn.6132	23.3	32.1	0.3	1.5	
Mouse dihydrofolate reductase gene, 3' flank, segment 2.	M13215	N.A.	N.A.	5.3	6.0	1.0	0.9	
Mouse lysyl hydroxylase isoform 2 mRNA, complete cds.	AF030572	NM_182943	N.A.	4.4	19.1	0.8	1.9	
KIAA1568 protein= ROBO2	BAB13394	N.A.	N.A.	4.0	3.6	1.1	0.4	

Figure 14C

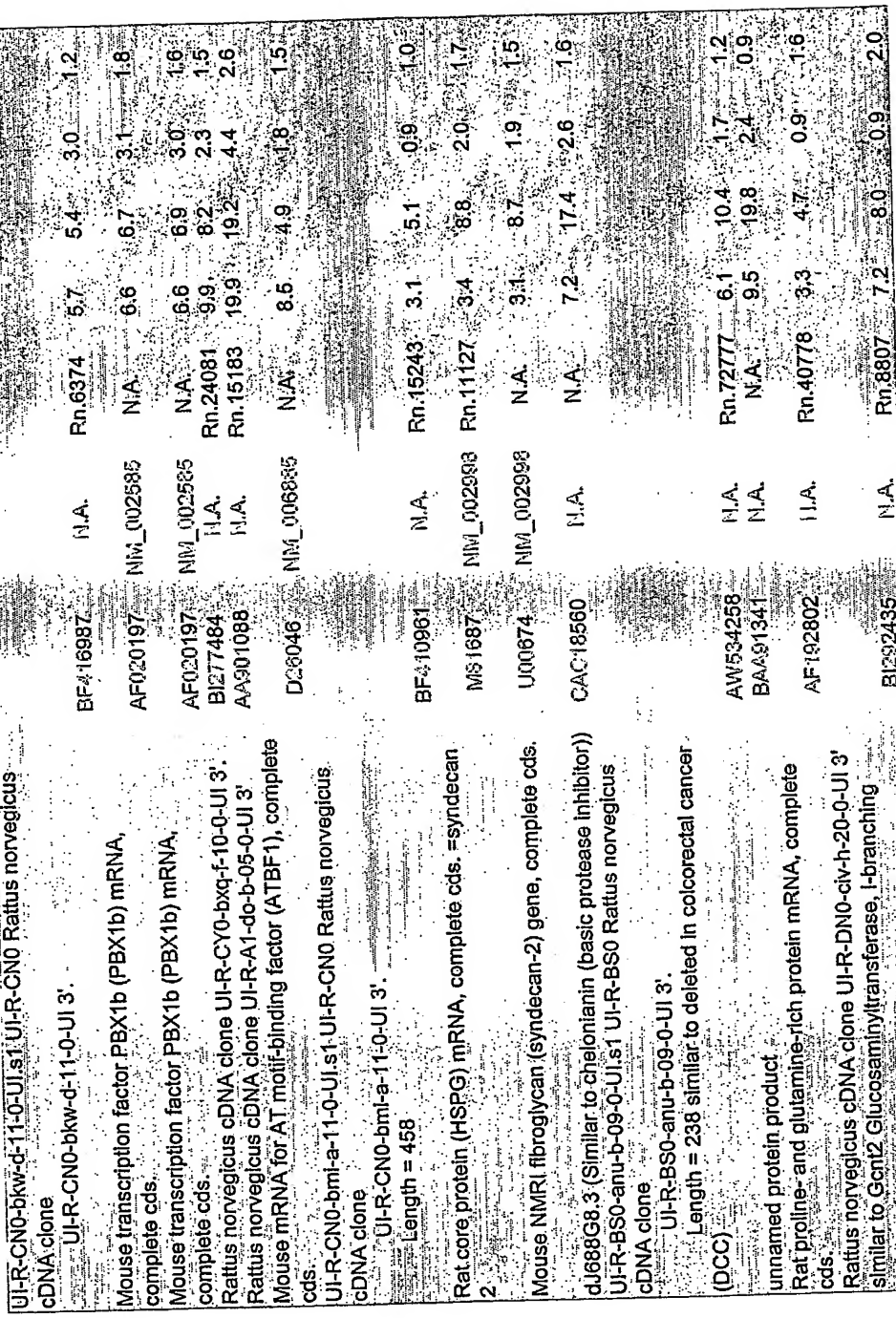
Mouse NAD(+)-dependent 15-hydroxyprostaglandin dehydrogenase mRNA, complete cds.	U44389	NM_000860	N.A.	8.7	7.8	0.5	0.3
Tumor-associated calcium signal transducer 1	AJ001044	NM_002354	Rn.24930	9.2	5.1	0.7	0.3
Ca2+ dependent activator protein for secretion	BC023929	NM_017954	N.A.	10.5	9.7	0.8	0.2
Rattus norvegicus cDNA clone UI-R-C2p-oc-a-05-0-UI 5'.	BF551935	N.A.	Rn.1135	8.2	5.9	0.5	0.4
Mouse retinal short-chain dehydrogenase/reductase 1 (Rsdr1) gene, exon 1.	AF179238	N.A.	N.A.	5.6	10.5	0.4	0.4
Mouse mRNA for caspase-7.	Y13088	NM_033339	N.A.	11.5	10.3	0.7	0.5
Rattus norvegicus cDNA clone UI-R-DK0-cej-b-12-0-UI 3'.	BI294080	N.A.	Rn.1135	8.3	8.0	0.8	0.6
Rat mRNA for protein kinase PASK, complete cds.	D88190	NM_013233	Rn.15731	4.0	4.4	1.1	0.5
Rat mRNA for protein kinase PASK, complete cds.	D88190	NM_013233	Rn.15731	4.1	4.5	1.2	0.5
Mouse encephalopsin mRNA, complete cds.	AF140241	NM_001821	N.A.	5.8	8.3	1.1	0.7
Mouse laminin beta3 (Lamb3) chain mRNA, complete cds.	U43298	NM_000228	N.A.	12.3	22.3	1.4	0.2
Rattus norvegicus cDNA clone UI-R-BT1-bnq-f-02-0-UI 3'.	BF412664	N.A.	Rn.28067	5.2	2.8	0.5	0.9
Mus musculus glucan (1,4-alpha-), branching enzyme 1 (Gbe1),	NM_028803	NM_000158	Mm.29201	4.5	3.6	0.8	1.2
UI-R-BJ0-aec-c-12-0-UI.s1 UI-R-BJ0 Rattus norvegicus cDNA clone UI-R-BJ0-aec-c-12-0-UI 3'.	AW253868	N.A.	Rn.41237	6.5	4.0	0.6	3.6
Rattus norvegicus glycoprotein hormone alpha 2 (Gpha2), mRNA	NM_133619	NM_130769	Rn.22078	8.0	5.6	0.4	2.2
Mus musculus adult male olfactory brain cDNA, RIKEN full-length enriched library, clone:6430550H21 product:HYPOTHETICAL 38.5 KDA PROTEIN homolog	AK078264	N.A.	N.A.	24.5	29.3	6.5	11.3
Mouse Dpf11 mRNA for polyposis locus protein 1-like 1 (TB2 protein-like 1), complete cds.	AB039933	NM_138393	N.A.	10.5	4.1	1.6	1.3
Mouse chromosome 17 clone GS-384N12, complete sequence. nedd4???	AC087217	N.A.	N.A.	11.8	6.9	1.6	2.3
Rat glutamate receptor interacting protein 2 mRNA, complete cds.	AF205193	AB051506	Rn.15680	9.3	7.8	2.2	2.4
UI-R-C3-td-e-06-0-UI.s1 UI-R-C3 Rattus norvegicus cDNA clone UI-R-C3-td-e-06-0-UI 3'.	AI548358	N.A.	Rn.41691	7.4	6.6	1.3	2.0

Figure 14D



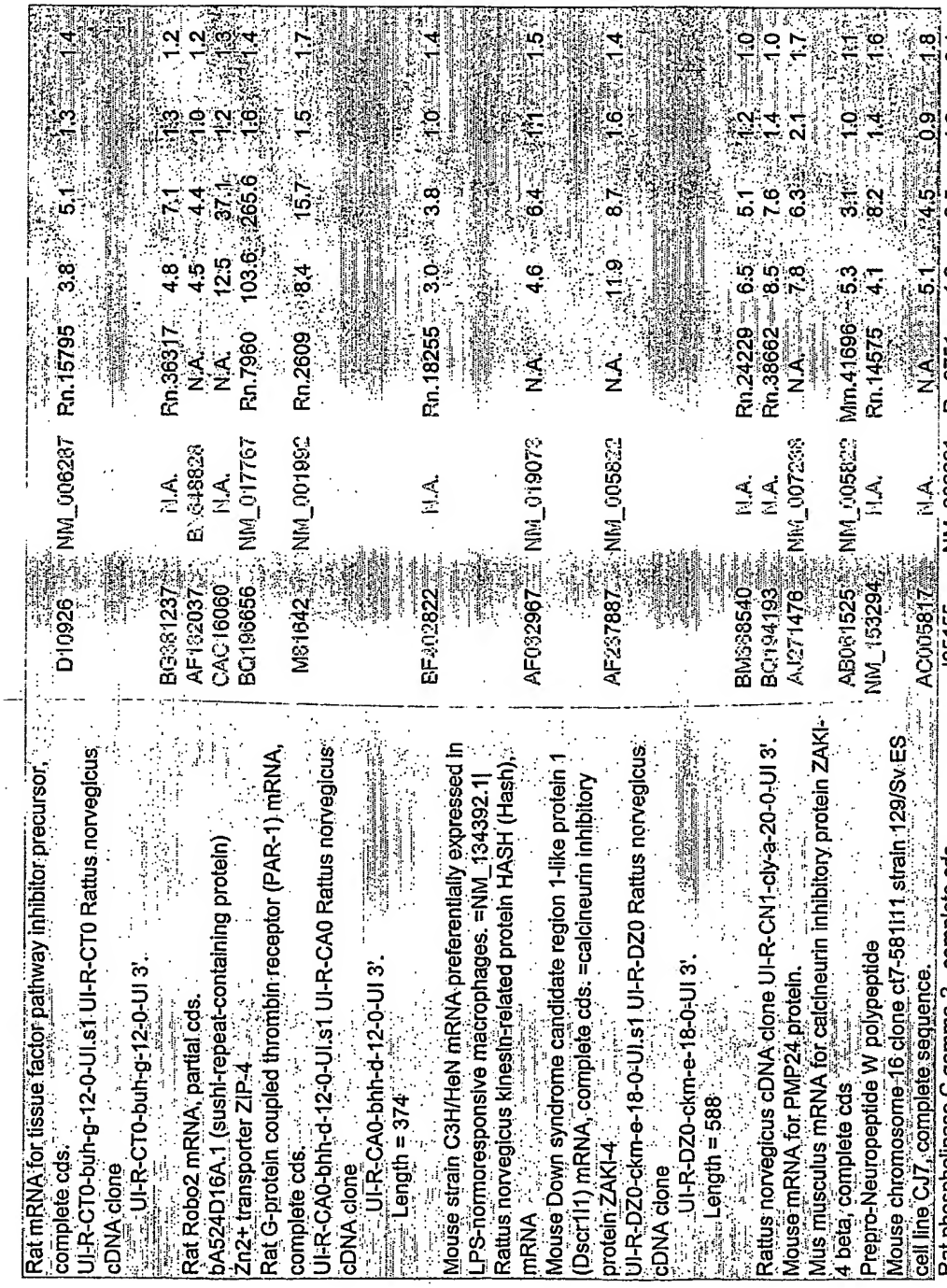
Rattus norvegicus cDNA clone UI-R-A0-bh-h-03-0-UI 3' similar H.sapiens mRNA for IL13 receptor alpha-1 chain.	AA819589	N.A.	Rn.2142	10.6	8.8	1.5	4.9		
Rattus norvegicus cDNA clone UI-R-DK0-cez-g-04-0-UI 3' Weakly similar to Notch 3 [Rattus norvegicus]	BI296384	N.A.	Rn.21560	24.8	16.1	1.3	5.5		
Rat interferon-inducible protein 16 mRNA, complete cds.	AF194040	N.A.	Rn.2648	5.8	6.7	1.6	2.9		
Mouse cyclin ania-6b gene, partial sequence.	AF185591	N.A.	N.A.	18.0	13.9	2.6	6.5		
Mouse fibroblast growth factor 1 gene, exon 3, complete sequence; and partial cds.	AF057190	N.A.	N.A.	28.0	11.2	0.8	4.9		
Mouse DHFR gene, exon 3.	V00737	N.A.	N.A.	8.7	16.7	1.5	2.7		
Rattus norvegicus cDNA clone UI-R-E1-fd-f-03-0-UI 5'. Similar to RNA binding motif, single stranded interacting protein 3; RNA-binding protein [Homo saplens]	BF561068	N.A.	Rn.8943	10.0	9.8	0.8	3.2		
Mouse p53 apoptosis-associated target (Perp) mRNA, complete cds.	AF249870	NM_022121	N.A.	13.1	9.4	0.7	4.5		
Rat p65 mRNA	X62772	N.A.	N.A.	5.2	4.4	1.0	2.4		
[Mus musculus RIKEN cDNA 1300007F04 gene (1300007F04Rik), mRNA									
Length = 2662									
	XM_126132	N.A.	Mm.258471	6.2	5.4	1.0	1.7		
Rattus norvegicus glutathione peroxidase 2 (Gpx2), mRNA	NM_183403	NM_002083	Rn.3503	51.0	13.3	1.3	2.8		
Mouse capping protein alpha 1 subunit mRNA, partial cds.	U16740	NM_006135	N.A.	4.4	4.7	2.6	1.0		
UI-R-DO1-cms-k-22-0-UI.s1 UI-R-DO1 Rattus norvegicus cDNA clone									
UI-R-DO1-cms-k-22-0-UI 3'.	BQ202872	N.A.	Rn.22181	3.9	4.8	2.0	0.8		
AMGNNUC:NRHY5-00457-A9-A W Rat hypothalamus (10471) Rattus norvegicus cDNA clone nrhy5-00457-a9 5'.	CB695344	N.A.	Rn.46995	9.4	8.9	3.4	0.5		
UI-R-DK0-cfw-b-04-0-UI.s1 UI-R-DK0 Rattus norvegicus cDNA clone									
UI-R-DK0-cfw-b-04-0-UI 3'. Similar to Solute carrier family 21 (organic anion transporter), member 12									
	BI290422	N.A.	Rn.8208	5.7	6.9	2.1	1.0		

Figure 14E



UI-R-CN0-bkw-d-11-0-UI.s1 UI-R-CN0 Rattus norvegicus cDNA clone										
UI-R-CN0-bkw-d-11-0-UI 3'.	BF416987	N.A.	Rn.6374	5.7	5.4	3.0	1.2			
Mouse transcription factor PBX1b (PBX1b) mRNA, complete cds.	AF020197	NM_002585	N.A.	6.6	6.7	3.1	1.8			
Mouse transcription factor PBX1b (PBX1b) mRNA, complete cds.	AF020197	NM_002585	N.A.	6.6	6.9	3.0	1.6			
Rattus norvegicus cDNA clone UI-R-CY0-bxq-f-10-0-UI 3'.	BI277464	N.A.	Rn.24081	9.9	8.2	2.3	1.5			
Rattus norvegicus cDNA clone UI-R-A1-do-b-05-0-UI 3'	AA901088	N.A.	Rn.15183	19.9	19.2	4.4	2.6			
Mouse mRNA for AT motif-binding factor (ATBF1), complete cds.	D26046	NM_006885	N.A.	8.5	4.9	1.8	1.5			
UI-R-CN0-bmi-a-11-0-UI.s1 UI-R-CN0 Rattus norvegicus cDNA clone										
UI-R-CN0-bmi-a-11-0-UI 3'. Length = 458	BF410961	N.A.	Rn.15243	3.1	5.1	0.9	1.0			
Rat core protein (HSPG) mRNA, complete cds. =syndecan 2	M91687	NM_002998	Rn.11127	3.4	8.8	2.0	1.7			
Mouse NMRI fibroglycan (syndecan-2) gene, complete cds.	U00674	NM_002998	N.A.	3.1	8.7	1.9	1.5			
dJ688G8.3 (Similar to chelonianin (basic protease inhibitor))	CAC18560	N.A.	N.A.	7.2	17.4	2.6	1.6			
UI-R-BS0-anu-b-09-0-UI.s1 UI-R-BS0 Rattus norvegicus cDNA clone										
UI-R-BS0-anu-b-09-0-UI 3'. Length = 238 similar to deleted in colorectal cancer (DCC)	AW534258	N.A.	Rn.72777	6.1	10.4	1.7	1.2			
unnamed protein product	BAA91341	N.A.	N.A.	9.5	19.8	2.4	0.9			
Rat proline- and glutamine-rich protein mRNA, complete cds.	AF192802	N.A.	Rn.40778	3.3	4.7	0.9	1.6			
Rattus norvegicus cDNA clone UI-R-DN0-civ-h-20-0-UI 3' similar to Gcnt2 Glucosaminyltransferase, I-branching enzyme	BI292435	N.A.	Rn.6807	7.2	8.0	0.9	2.0			

Figure 14F



Rat mRNA for tissue factor pathway inhibitor precursor, complete cds.	D10926	NM_006287	Rn.15795	3.8	5.1	1.3	1.4			
UI-R-CT0-buh-g-12-0-UI.s1 UI-R-CT0 Rattus norvegicus cDNA clone										
UI-R-CT0-buh-g-12-0-UI 3'.										
	BG381237	N.A.	Rn.36317	4.8	7.1	1.3	1.2			
Rat Robo2 mRNA, partial cds.	AF162037	BC048828	N.A.	4.5	4.4	1.0	1.2			
bA524D16A.1 (sushi-repeat-containing protein)	CAC16060	N.A.	N.A.	12.5	37.1	1.2	1.3			
Zn2+ transporter ZIP-4	BC106656	NM_017767	Rn.7960	103.6	265.6	1.6	1.4			
Rat G-protein coupled thrombin receptor (PAR-1) mRNA, complete cds.	M81842	NM_001992	Rn.2609	8.4	15.7	1.5	1.7			
UI-R-CA0-bhh-d-12-0-UI.s1 UI-R-CA0 Rattus norvegicus cDNA clone										
UI-R-CA0-bhh-d-12-0-UI 3'.										
Length = 374										
	BF402822	N.A.	Rn.18255	3.0	3.8	1.0	1.4			
Mouse strain C3H/HeN mRNA preferentially expressed in LPS-normoresponsive macrophages. =NM_134392.1] Rattus norvegicus kinesin-related protein HASH (Hash), mRNA	AF032967	NM_019073	N.A.	4.6	6.4	1.1	1.5			
Mouse Down syndrome candidate region 1-like protein 1 (Dscr1l1) mRNA, complete cds. =calcineurin inhibitory protein ZAKI-4	AF237887	NM_005822	N.A.	11.9	8.7	1.6	1.4			
UI-R-DZ0-ckm-e-18-0-UI.s1 UI-R-DZ0 Rattus norvegicus cDNA clone										
UI-R-DZ0-ckm-e-18-0-UI 3'.										
Length = 588										
	BM388540	N.A.	Rn.24229	6.5	5.1	1.2	1.0			
Rattus norvegicus cDNA clone UI-R-CN1-cly-a-20-0-UI 3'.	BQ194193	N.A.	Rn.38662	8.5	7.6	1.4	1.0			
Mouse mRNA for PMP24 protein.	AJ271478	NM_007268	N.A.	7.8	6.3	2.1	1.7			
Mus musculus mRNA for calcineurin inhibitory protein ZAKI-4 beta, complete cds	AB061525	NM_005822	Mm.41696	5.3	3.1	1.0	1.1			
Prepro-Neuropeptide W polypeptide	NM_153294	N.A.	Rn.14575	4.1	8.2	1.4	1.6			
Mouse chromosome 16 clone ctf-581i11 strain 129/Sv ES cell line CJ7, complete sequence.	AC005817	N.A.	N.A.	5.1	4.5	0.9	1.8			
Rat phospholipase C gamma-2 , complete cds.	J05155	NM_002661	Rn.9751	1.2	8.5	1.0	0.4			

Figure 14G

Rat kidney extracellular calcium-sensing receptor mRNA, complete cds.	U10354	NM_000388	Rn.10019	0.9	16.8	0.9	0.2	
Rat follistatin gene, exon 6, clones pROF(301-305).	M31591	N.A.	N.A.	0.6	10.5	1.9	0.4	
Mouse CDO (Cdo) mRNA, complete cds.	AF090866	NM_016952	N.A.	0.6	2.5	1.0	0.3	
Mouse mRNA for collagen type XIV, partial.	AJ131395	In	N.A.	0.5	2.5	1.1	0.3	
PMEPA1 protein	AAF86322	N.A.	N.A.	3.7	1.7	0.6	3.3	
SKAP55	CAA72101	N.A.	N.A.	12.4	2.6	0.9	13.5	
Rattus norvegicus cDNA clone UI-R-DM1-ckc-l-19-0-UI 3'.	BM391951	N.A.	Rn.57622	6.2	2.7	1.3	9.1	
KIAA0967 protein	BAA76811	N.A.	N.A.	3.3	3.9	1.0	5.5	
Mouse chromosome 17 clone GS1-11M9 strain 129svj, complete sequence.	AC087216	N.A.	N.A.	5.9	3.2	1.1	6.2	
Rat mRNA for sensory neuron synuclein.	X86789	NM_003087	Rn.10421	28.1	4.3	1.8	29.5	
Mouse serine protease inhibitor (SPI3) mRNA, complete cds.	U25844	N.A.	N.A.	7.3	5.2	2.2	9.0	
Rat lens mRNA for aldose reductase (AR,EC 1.1.1.21).	X05884	N.A.	Rn.2917	4.0	5.0	2.4	11.3	
Mouse chromosome 10 popeye protein 3 (Pop3) mRNA, complete cds.	AF204176	N.A.	N.A.	5.8	4.1	1.8	10.2	
Mouse mRNA for T-cadherin, complete cds.	AB022100	NM_001257	N.A.	5.7	5.7	1.4	11.0	
FK506 binding protein precursor	AAF63478	N.A.	N.A.	3.3	2.4	1.2	6.4	
Mouse partial mRNA for dysferlin (dysf gene).	AJ242954	NM_003494	N.A.	6.3	4.3	1.8	25.9	
Mouse Eya2 homolog mRNA, partial cds.	U61111	NM_172113	N.A.	2.8	5.6	0.2	54.1	
polyprotein	AAG10201	N.A.	N.A.	1.6	1.3	0.9	6.0	
LC NA CLUSTER 1								
transcribed sequence with moderate similarity to protein ref:NP_001227.1 (H.sapiens) carbonyl reductase 3	BQ206123	N.A.	Rn.8624	0.6	1.5	1.5	4.3	
similar to AD037 protein containing RalGDS/AF-6 domain	BI293840	N.A.	N.A.	0.5	1.4	1.0	5.0	
similar to AD037 protein containing RalGDS/AF-6 domain	XM_232305	N.A.	N.A.	0.6	1.5	1.0	5.8	
axonemal dynein heavy chain	CAB94756	N.A.	N.A.	1.0	2.6	1.0	4.0	
Rat inhibitor of apoptosis protein 1 mRNA, complete cds.	AF183430	NM_001166	Rn.64578	2.2	5.6	1.8	13.0	
Cricetulus griseus laylin mRNA, complete cds.	AF093673	N.A.	N.A.	1.3	0.7	1.2	6.9	
Rattus norvegicus cDNA clone UI-R-DS0-cje-d-16-0-UI 3'	BM383125	N.A.	Rn.43431	1.3	3.4	1.0	28.9	
neuronal tryptophane hydroxylase	NM_173839	N.A.	N.A.	1.8	1.5	1.3	6.4	

Figure 14H

Rattus norvegicus cDNA clone UI-R-C2p-qn-b-09-0-UI 3' 45nt similar to hypothetical Eukaryotic thiol (cysteine) proteases active site containing protein	AI555069	N.A.	Rn.17968	1.5	2.0	1.5	11.4			
Rat rabphilin-3a related protein mRNA, complete cds.	AF022774	NM_006867	Rn.10986	1.1	2.0	1.6	5.7			
Rattus norvegicus neuroglobin (Ngb), mRNA.	NM_033359	NM_021257	Rn.64845	1.8	2.6	6.9	65.8			
cytochrome b-561 (Cyb561)	NP_031831	N.A.	Rn.14673	1.4	1.6	2.7	28.3			
Rattus norvegicus cDNA clone UI-R-A0-bf-d-10-0-UI 3' similar to emb|X60819|HSMAOP14 H.sapiens DNA for monoamine oxidase type A (14)	AA858657	NM_000240	Rn.16867	1.9	2.5	3.0	16.9			
Rat Guanine nucleotide-binding protein G(S), alpha subunit (Adenylate cyclase-stimulating G alpha protein).	AF107845	NM_080425	Rn.31	1.3	2.6	2.5	21.0			
Rattus norvegicus LOC299269 (LOC299269), mRNA										
weakly similar to interferon, alpha-inducible protein 27-like	XM_238467	N.A.	Rn.16842	1.7	1.4	2.2	24.9			
Rat copper transporter 1 mRNA, complete cds.	AF268030	NM_001859	Rn.2789	2.0	2.3	1.7	39.1			
Rattus norvegicus cDNA clone UI-R-EA0-ckw-i-06-0-UI 3' weakly similar to CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	BQ196724	N.A.	Rn.27228	1.5	1.7	1.2	13.0			
Mouse Ccte gene for chaperonin containing TCP-1 epsilon subunit, complete cds.	AB022158	N.A.	N.A.	2.6	2.0	1.6	100.6			
Rattus norvegicus cDNA clone RGIDW96	AW916943	N.A.	Rn.91509	1.2	1.3	5.0	#DIV/0!			
Rattus norvegicus cDNA clone UI-R-C1-lm-g-11-0-UI 5'.	BF559920	N.A.	Rn.19423	1.9	2.3	1.8	22.5			
Rat mama mRNA, complete cds. (=PEPTIDYLPROLYL ISOMERASE C-ASSOCIATED PROTEIN)										
collectin 34	AF065438	N.A.	Rn.3251	2.0	3.0	1.6	7.8			
Rattus norvegicus cDNA clone UI-R-CN1-cjg-c-10-0-UI 3'.	BAA81747	N.A.	N.A.	2.2	3.8	2.3	20.3			
Rat mRNA for Rab3B protein.	BM386534	N.A.	Rn. 54190	0.7	1.8	2.2	4.6			
Rattus norvegicus cDNA clone UI-R-DK0-ceg-g-09-0-UI 3' similar to hypothetical protein DKFZp564B1162 with RHOGAP domain	Y14019	NM_002867	Rn.3788	0.7	2.4	2.3	10.8			
Rat monoamine oxidase A gene, complete cds.	BI295380	N.A.	Rn.13264	0.9	2.0	1.5	4.1			
Rat monoamine oxidase A gene, complete cds.	D00688	N.A.	N.A.	0.8	1.5	1.7	8.3			
poly(rC)-binding protein 3 (Pcbp3)	D00688	N.A.	N.A.	0.8	1.2	1.8	11.8			
neuronal tetraspanin	AF176327	NM_020523	N.A.	1.6	1.0	0.9	4.4			
Rat fructose-1,6-bisphosphatase mRNA, complete cds.	AAF19031	N.A.	N.A.	0.9	1.4	0.9	9.5			
Rat dopamine beta-hydroxylase mRNA, complete cds.	M86240	NM_000507	Rn.33703	0.6	1.0	0.9	35.4			
	L12407	NM_000787	Rn.10912	0.9	0.7	0.8	593.9			

Figure 14I

Mouse pigment epithelium-derived factor (PEDF) mRNA, complete cds.	AF036164	NM_002615	N.A.	1.0	1.1	1.0	12.2			
Rat dopamine beta-hydroxylase mRNA, complete cds.	L12407	NM_000787	Rn.10912	1.3	1.0	1.1	31.4			
Mouse RIBP (Ribp) mRNA, complete cds.	AF203343	NM_003975	N.A.	1.0	1.4	1.5	23.7			
Rat amino acid transporter y+LAT1 mRNA, complete cds.	AF200684	NM_003982	Rn.18058	1.7	1.0	2.1	5.1			
palate, lung, and nasal epithelium expressed transcript (Plunc)	U69172	NM_130852	N.A.	0.6	9.1	6.7	5.3			
Rat PND gene encoding atrial natriuretic factor, complete cds.	K02062	N.A.	Rn.2004	0.7	1.7	11.1	2.8			
Rat PND gene encoding atrial natriuretic factor, complete cds.	K02062	N.A.	Rn.2004	0.5	1.8	14.1	2.9			
Rattus norvegicus cDNA clone UI-R-CV1-brw-a-04-0-UI 3' weakly similar to gamma-aminobutyric acid (GABA-A) receptor, subunit epsilon	BG373206	N.A.	Rn.17221	0.9	1.3	28.2	6.7			
10-formyltetrahydrofolate dehydrogenase	AAA70429	N.A.	N.A.	1.3	1.9	8.0	4.1			
Rat (Sprague-Dawley) mRNA for growth hormone-releasing hormone -like peptide.	Z34092	NM_021081	N.A.	1.8	1.7	133.6	21.3			
Rat CART protein mRNA, complete cds.	U10071	NM_004291	Rn.10201	0.4	0.6	19.6	5.2			
10-formyltetrahydrofolate dehydrogenase	AAA70429	N.A.	N.A.	0.9	1.0	7.3	3.7			
Rattus norvegicus cDNA clone UI-R-C4-alq-h-05-0-UI 3'.	AW534235	N.A.	Rn.68154	0.6	1.8	3.2	6.8			
ONECUT-2 transcription factor (OC-2)	CAB38253	N.A.	N.A.	0.6	0.8	4.5	7.2			
Rat mRNA for glucokinase, alternatively spliced GK2 (EC 2.7.1.1).	X53588	NM_000162	Rn.10447	0.7	1.4	32.7	41.8			
Miwi 2 protein (Miwi2)	AY135692	N.A.	N.A.	1.1	1.5	5.3	14.5			
Mus musculus cDNA clone UI-M-DJ2-bwb-j-08-0-UI 3' dJ620E11.2 (novel protein similar to KIAA0188, KIAA0249 and yeast SMP2)	BQ174977	N.A.	Mm.153072	0.5	1.0	3.9	13.4			
Mouse C1q-related factor mRNA, complete cds.	CAB57842	N.A.	N.A.	0.8	1.6	3.5	3.6			
Rattus norvegicus cDNA clone UI-R-BT1-akv-g-05-0-UI 3'.	AF095155	N.A.	N.A.	1.2	3.3	4.8	7.5			
Rattus norvegicus cDNA clone UI-R-C0-hi-d-02-0-UI 3' similar to Necdin-like protein 1 and melanoma antigen, family L, 2 (Magel2), involved in Prader-Willi syndrome	AW531194	N.A.	Rn.46621	1.1	3.5	7.3	6.0			
Rat huntingtin associated protein (rHAP1-B) mRNA, complete cds, alternatively spliced form.	AA996569	N.A.	N.A.	1.6	5.3	10.8	4.2			
	U38370	NM_003949	Rn.37430	1.3	3.7	8.2	5.5			

Figure 14J

Rat mRNA for plasma glutathione peroxidase precursor, complete cds.	D00680	NM_002084	Rn.1491	5.2	7.4	16.3	40.7		
unnamed protein product	BAA91387	N.A.	N.A.	2.2	2.2	2.8	8.5		
Mouse ectonucleoside triphosphate diphosphohydrolase 3	AK046218	NM_001248	N.A.	3.3	2.2	3.4	10.9		
KIAA0605 protein protein hypothetical Thrombospondin type I repeat (TSP1) profile/Thrombospondin type I domain containing protein	BAA25531	N.A.	N.A.	2.4	2.7	15.2	39.2		
Mouse neural retina-specific leucine zipper protein (NRL) mRNA, complete cds.	L14935	NM_006177	N.A.	1.4	1.8	3.1	6.3		
Rat synapsin 2a mRNA, complete cds.	M27925	NM_003178	Rn.506	0.8	0.6	1.7	4.1		
LC NA CLUSTER 2									
Mouse protein tyrosine kinase ZAP-70 mRNA, complete cds.	U04379	NM_001079	N.A.	0.4	0.7	2.1	7.5		
Rattus norvegicus regucalcin gene promotor region related protein (Rgpr), mRNA	NM_053571	NM_033127	Rn.21873	0.2	0.8	1.5	3.5		
Rattus norvegicus cDNA clone UI-R-BO0-ahn-f-03-0-UI 3'.	AW532862	N.A.	Rn.4597	0.4	1.2	0.3	5.3		
Rattus norvegicus cDNA clone UI-R-CW0-bvx-g-01-0-UI 3'.	BG371623	N.A.	Rn.23870	0.4	0.4	0.9	3.8		
Mouse TNF receptor associated factor 1 (TRAF1) mRNA, complete cds.	L35302	NM_005658	N.A.	0.7	0.7	1.0	5.0		
Rat PGES mRNA for prostaglandin E synthase, complete cds.	AB041998	NM_198797	Rn.7730	0.5	1.0	1.2	4.0		
Rat gamma glutamyl transpeptidase-related enzyme mRNA, partial cds.	U76252	NM_004121	Rn.44367	0.4	0.8	1.2	10.8		
Rat mRNA for Ig heavy chain VDJ-region CH1-CH2.	X66782	N.A.	N.A.	0.4	0.6	0.8	5.0		
unnamed protein product, no homology	BAA91309	N.A.	N.A.	0.4	0.5	0.9	18.9		
Rat gelatinase A mRNA, complete cds.	U65656	NM_004530	Rn.6422	0.8	0.7	0.3	3.6		
Rattus norvegicus cDNA clone UI-R-CA1-bbw-h-04-0-UI 3'.	BF557974	N.A.	Rn.43361	0.7	0.6	0.2	3.9		
Rattus norvegicus cDNA clone UI-R-BT1-ako-f-11-0-UI 5'.	BF565662	N.A.	Rn.46917	0.5	0.6	0.3	10.5		
Similar to sodium-dependent bile acid transporter	AW533438	N.A.	Rn.40871	0.9	0.4	0.3	7.5		
Rattus norvegicus cDNA clone UI-R-BU0-anb-h-08-0-UI 3'.									
Weakly similar to zinc finger protein, multitype 1; Friend of GATA-1 [Mus musculus]									

Figure 14K

Rat mRNA for l-gicerin/MUC18, complete cds.	AB035506	NM_006500	Rn.64570	0.7	0.7	0.4	4.3	
Rattus norvegicus copper transporter 1 mRNA, complete cds.AF268030	AF268030	NM_001859	N.A.	0.6	0.5	0.6	7.4	
Mus musculus transcription factor AP-2 beta, full insert sequence	AK028906	N.A.	Mm.4795	0.3	0.4	0.4	19.2	
Rat mRNA for long-chain acyl-CoA synthetase (EC 6.2.1.3).	D90109	NM_001995	Rn.6215	0.6	0.8	0.8	4.9	
unnamed protein product	BAB14064	N.A.	N.A.	0.3	0.3	0.5	67.4	
Rat beta-alanine synthase mRNA, complete cds.	M97662	NM_016327	Rn.11110	0.4	0.4	0.6	6.8	
Mitochondrial uncoupling protein 3 UCP3	U92069	NM_003356	Rn.9902	0.8	0.8	0.7	4.5	
Rattus norvegicus cDNA clone UI-R-C3-te-e-09-0-UI 3'.	AI548631	N.A.	Rn.22936	0.3	0.4	0.4	5.8	
Mouse pigment epithelium-derived factor (PEDF) mRNA, complete cds.	AF036164	NM_002615	N.A.	0.1	0.2	0.2	12.7	
Rat alpha 1 type V collagen mRNA, complete cds.	AF272662	NM_000093	Rn.117	0.4	0.6	0.3	5.5	
Rattus norvegicus cDNA clone UI-R-CV2-cht-d-08-0-UI 3'.	BI299288	N.A.	Rn.51523	0.3	0.5	0.3	6.9	
Rat aldehyde dehydrogenase mRNA, complete cds.	J03637	NM_000691	Rn.9810	0.6	0.6	0.7	12.0	
Rat fibrillin-1 mRNA, complete cds.	AF135059	NM_000138	Rn.12759	0.5	1.7	0.9	3.5	
Rat adenylyl cyclase mRNA, complete cds.	M80550	NM_020546	Rn.10731	0.2	0.4	1.1	2.5	
Rat degenerin channel MDEG mRNA, complete cds.	U53211	NM_183377	Rn.37523	0.2	0.2	1.4	2.5	
unnamed protein product	BAB15639	N.A.	N.A.	0.3	0.6	1.2	1.5	
KIAA0808 protein	BAA34528	N.A.	N.A.	0.1	0.3	0.8	2.2	
PNG protein	CAA66121	N.A.	N.A.	0.3	0.2	1.0	2.0	
Mouse CD40 mRNA, complete cds.	M83312	NM_001250	N.A.	0.2	0.4	0.4	2.5	
Mouse EIG 180 mRNA for ethanol induced gene product, complete cds.	AB023957	N.A.	N.A.	0.2	0.7	0.5	3.0	
Rat heat stable antigen CD24 mRNA, complete cds.	U49062	NM_013230	Rn.6007	0.1	0.5	0.3	3.5	
Rattus norvegicus cDNA clone UI-R-DK0-cez-c-07-0-UI 3'.	BI296344	N.A.	Rn.46362	0.3	0.2	0.6	2.2	
Rat beta-alanine synthase mRNA, complete cds.	M97662	NM_016327	Rn.11110	0.1	0.2	0.4	6.4	
Rattus norvegicus cDNA clone UI-R-BJ0-aeo-b-12-0-UI 3'.	AW254017	NM_000093	Rn.40162	0.2	0.3	0.1	2.9	
chromatin remodeling factor WCRF180	AAF32366	N.A.	N.A.	0.2	0.2	0.4	2.5	
Mouse mRNA for IGFBP-like protein, complete cds.	AB006141	N.A.	N.A.	0.2	0.3	1.5	1.3	
Rattus norvegicus cDNA clone UI-R-CV0-brm-d-06-0-UI 3'.	BG372104	N.A.	Rn.22267	0.1	0.3	1.0	1.2	
Rat Edg-1 orphan receptor (edg-1) mRNA, complete cds.	U10303	NM_001400	Rn.4102	0.2	0.4	1.0	0.8	

Figure 14L

Rat neuronal olfactomedin-related ER localized protein (D2Sut1e) mRNA, complete cds.	U03417	NM_014279	Rn.11005	0.1	0.4	0.7	0.9
Rat glucocorticoid receptor mRNA, complete cds.	M14053	NM_000176	Rn.29814	0.2	0.4	0.6	1.1
Rat glutathione S-transferase mRNA, complete cds.	J03752	NM_145791	Rn.2580	0.2	0.2	0.5	1.3
Rat hepatic product spot 14, gene and flanks.	K01934	N.A.	Rn.9854	0.1	0.1	0.5	0.7
Cricetinae gen. sp. insulin receptor tyrosine kinase 53 kDa substrate mRNA, complete cds.	U41899	N.A.	N.A.	0.1	0.1	0.5	1.0
Rat myr 6 myosin heavy chain mRNA, complete cds.	U60416	AB032945	Rn.10640	0.1	0.1	0.9	0.8
Rat mRNA for pre-alpha-inhibitor, heavy chain 3.	X83231	N.A.	Rn.10391	0.2	0.3	0.7	1.0
unnamed protein product	BAA92093	N.A.	N.A.	0.2	0.3	0.9	1.0
Mouse mas proto-oncogene and Igf2r gene for insulin-like growth factor type 2 and L41ps and Au76 pseudogenes.	AJ249895	N.A.	N.A.	0.1	0.3	0.4	0.8
Rat mRNA for RT1.B-1(alpha) chain of integral membrane protein.	X14879	N.A.	Rn.25717	0.1	0.3	0.1	1.0
Rat mRNA for MHC class II-associated invariant chain.	X14254	N.A.	N.A.	0.1	0.1	0.1	1.1
Rat (diabetic BB) MHC class II alpha chain RT1.D alpha (u).	Y00480	N.A.	Rn.4200	0.1	0.1	0.1	0.9
Mouse clone 41 developing liver stage specifc mRNA.	U06946	N.A.	N.A.	0.2	0.3	0.3	1.2
Rat xanthine dehydrogenase mRNA, complete cds.	J05579	NM_000379	Rn.7324	0.2	0.3	0.3	1.5
Rat xanthine dehydrogenase mRNA, complete cds.	J05579	NM_000379	Rn.7324	0.2	0.2	0.3	1.4
Rat cardiac troponin T mRNA, complete cds, clone RCT11.	M26051	NM_000364	Rn.9965	0.1	0.1	0.1	2.9
Rat neuropeptide Y mRNA, complete cds.	M15880	NM_000905	Rn.9714	0.0	0.1	0.1	4.4
Rat m1 muscarinic acetylcholine receptor gene.	M16406	N.A.	Rn.44498	0.1	0.1	0.2	1.4
Mouse mRNA for src-like adaptor protein.	AJ131777	NM_006748	N.A.	0.0	0.1	0.0	0.7
Rat fos-related antigen DNA, exon 4.	X98051	N.A.	N.A.	0.1	0.1	0.1	0.4
Rat mRNA for muscle C-protein.	X90475	NM_002465	N.A.	0.1	0.1	0.1	0.4
Mouse mRNA for estrogen-responsive finger protein, complete cds.	D63902	N.A.	N.A.	0.2	0.2	0.2	0.8
Rat mRNA for cyclin B.	X64589	NM_031966	Rn.9232	0.3	0.2	0.3	1.1
Mouse ataxin 2-binding protein (A2bp) mRNA, complete cds.	AF107204	NM_145893	N.A.	0.1	0.1	0.2	1.0
Mouse ataxin 2-binding protein (A2bp) mRNA, complete cds.	AF107204	NM_145893	N.A.	0.1	0.1	0.1	0.6
Rat heat shock protein 27 (hsp 27) gene, complete cds.	S67755	N.A.	Rn.3841	0.1	0.2	0.1	0.6

Figure 14M

Mouse major histocompatibility locus class III regions Hsc70t gene, partial cds; smRNP, G7A, NG23, MutS homolog, CLCP, NG24, NG25, and NG26 genes, complete cds; and unknown genes.	AF109905	N.A.	N.A.	0.3	0.5	0.2	1.9		
Rat connexin (CXN-37) gene, complete cds.	M76532	N.A.	Rn.23406	0.3	0.3	0.3	1.6		
Rat pituitary adenylate cyclase activating polypeptide precursor protein mRNA, complete cds.	M63006	NM_001117	Rn.37400	0.2	4.0	0.3	1.4		
Neuroblastoma	BAA13745	N.A.	N.A.	0.9	0.3	2.4	0.2		
A13 DA CLUSTER 1									
Rat mRNA for hepatocyte nuclear factor 6 alpha.	X96553	N.A.	Rn.48812	0.1	0.5	27.4	0.3		
Rat vesicular GABA transporter (VGAT) mRNA, complete cds.	AF030253	NM_080552	Rn.10846	0.5	0.7	4.2	0.5		
Rat developmentally regulated intestinal protein (OCI-5) mRNA, complete cds.	M22400	NM_004484	Rn.9717	0.3	0.7	26.3	0.3		
Mus musculus LIM domain only 2 (rhombotin homolog 2)	A39370	N.A.	N.A.	0.4	0.6	15.5	0.4		
Mouse mRNA for Lim-homeodomain protein Islet1.	AJ132765	NM_002202	N.A.	0.4	0.3	21.4	0.2		
Rattus norvegicus cDNA clone UI-R-BS2-bdy-g-07-0-UI 3'.	BF396492	N.A.	Rn.58613	0.3	0.5	40.0	0.2		
UI-R-C4-aly-h-12-0-UI.s1 UI-R-C4 Rattus norvegicus cDNA clone UI-R-C4-aly-h-12-0-UI similar to HNF6 Hepatocyte nuclear factor 6 (HNF-6) (One cut domain family member 1)	AW535616	N.A.	Mm.227132	0.5	0.4	89.1	0.4		
Rat TTF-1 mRNA for thyroid nuclear factor 1.	X53958	NM_003317	Rn.34265	0.3	0.3	10.4	0.3		
Rat homeoprotein (rDlx) mRNA, complete cds.	L24443	NM_005221	Rn.11248	0.2	0.2	7.2	0.1		
Rattus norvegicus cDNA clone UI-R-C2p-gq-c-12-0-UI 3'.	AI555460	N.A.	Rn.9039	0.3	0.3	4.5	0.3		
Rattus norvegicus cDNA clone UI-R-BO1-ajs-b-09-0-UI 3'.									
Weakly similar to immunoglobulin superfamily containing leucine-rich repeat, (slit homolog 1?)	AW527248	N.A.	Rn.16271	0.1	0.1	8.2	0.1		
Rat enkephalinase (neutral endopeptidase) mRNA.	M15944	NM_007289	Rn.33598	0.2	0.3	8.9	0.2		
Rattus norvegicus cDNA clone UI-R-A1-dw-d-10-0-UI 3'	AA901371	N.A.	Rn.24326	0.7	1.2	3.3	0.3		
Mouse stanniocalcin precursor, mRNA, complete cds.	AF099098	NM_003155	N.A.	0.2	0.8	2.9	0.7		
Mouse retinaldehyde dehydrogenase 3 mRNA, complete cds.	AF253409	NM_000693	N.A.	0.9	0.7	7.1	0.4		
Rat mRNA for prepronociceptin.	X97375	NM_006228	Rn.11194	0.7	0.5	5.8	1.1		
Rat mRNA for vascular type-1 angiotensin II receptor.	X62295	N.A.	N.A.	0.2	1.2	5.2	0.1		

Figure 14N

Mouse phospholipid scramblase 1 mRNA, complete cds.	AF159593	NM_021105	N.A.	0.3	1.1	2.3	1.3	
putative secreted protein ZSIG9	AAF01433	N.A.	N.A.	1.9	4.1	0.1	0.2	
SN/VTA DA CLUSTER 2								
Mus musculus RIKEN cDNA 5430406M13 gene (5430406M13Rik), mRNA similar to mesenchymal stem cell protein DSC54	XM_128866	N.A.	N.A.	2.8	3.3	0.5	0.2	
Rat alpha-1B adrenergic receptor mRNA, complete cds.	M60655	NM_000679	Rn.10032	1.5	3.8	0.8	0.2	
Myopodin protein =synaptopodin-2	CAE51856	N.A.	N.A.	1.2	3.2	0.4	0.2	
Mus musculus 2 days neonate thymus thymic cells cDNA, RIKEN full-length enriched library, clone:E430007D18 product:special AT-rich sequence binding protein 1, full	AK008204	NM_002971	Mm.4381	2.0	1.5	0.4	0.2	
Mus musculus 2 days neonate thymus thymic cells cDNA, RIKEN full-length enriched library, clone:E430007D18 product:special AT-rich sequence binding protein 1, full	AK008204	NM_002971	Mm.4381	2.4	1.4	0.2	0.1	
Rat liver alpha-2-macroglobulin mRNA, complete cds.	J02635	NM_000014	Rn.780	2.0	1.9	0.5	0.2	
Mouse mRNA for cadherin-associated protein (CAP102/alpha catenin).	D90362	N.A.	N.A.	1.2	1.7	0.4	0.2	
CADHERIN 11	X77557	NM_053664	N.A.	1.6	2.1	0.5	0.2	
Mus musculus 15 days embryo head cDNA, RIKEN full-length enriched library, clone:D930020D06 product:forkhead box P2, full insert sequence	AK053022	NM_148900	Mm.133208	2.4	4.9	0.2	0.1	
Rat Sprague-Dawley SM-20 mRNA, complete cds.	U06713	NM_022073	Rn.10994	2.3	1.9	0.4	0.1	
C.griseus rhodopsin gene for opsin protein.	X61084	N.A.	N.A.	3.2	1.4	0.4	0.2	
Rattus norvegicus similar to hypothetical protein dJ434O14.3 (Homo sapiens) (LOC289387), mRNA	XM_213978	N.A.	N.A.	1.7	2.3	0.5	0.2	
Rattus norvegicus cDNA clone UI-R-CA1-bix-f-16-0-UI 3'	BF403670	N.A.	N.A.	3.7	2.8	0.2	0.3	
Rat mRNA for protein kinase C alpha.	X07286	N.A.	N.A.	3.4	1.1	0.2	0.5	
DRNBTE01 Rat DRG Library Rattus norvegicus cDNA clone DRNBTE01 5'.	BG673363	N.A.	N.A.	3.4	0.9	0.3	0.3	
Mouse TFPIbeta (TFPIbeta) mRNA, partial cds.	AF016313	NM_006287	N.A.	1.0	0.9	0.2	0.2	
Mouse transgelin mRNA, complete cds.	AF149291	NM_003564	N.A.	1.0	1.0	0.2	0.2	
Rat neuritin mRNA, complete cds.	U88958	N.A.	Rn.3546	1.5	1.1	0.1	0.4	

Figure 14O

Mouse ets-related transcription factor (Etv5) mRNA, complete cds.	AY004174	NM_004454	N.A.	1.7	1.3	0.2	0.3
Mouse mRNA for voltage gated calcium channel alpha-2-delta-C subunit.	AJ010949	NM_018398	N.A.	1.9	1.2	0.2	0.2
Mouse frazzled (fre) mRNA, complete cds.	U68058	NM_001463	N.A.	1.0	0.5	0.1	0.2
Mouse left-right dyneln (Lrd) mRNA, complete cds.	AF183144	N.A.	N.A.	0.9	0.8	0.2	0.3
insulin-like growth factor I (exon 6) [rats, Genomic/mRNA, 7224 nt].	S43941	N.A.	N.A.	2.9	1.2	0.9	0.2
Rat insulin-like growth factor I (IGF-I) mRNA, complete cds.	M15481	NM_000618	Rn.6282	4.1	1.5	0.9	0.1
Mus musculus, claudin 1, clone IMAGE:3493550, mRNA	BC008536	NM_021101	N.A.	0.5	2.0	0.3	0.4
Rat mRNA for LTBP-2 like protein.	Y12760	NM_000428	Rn.40921	1.5	1.5	0.2	0.7
Mouse GARP45 mRNA, complete cds.	AB018374	NM_030763	N.A.	0.8	1.3	0.6	0.2
Mouse mRNA for GATA-3 transcription factor.	X55123	NM_002051	N.A.	0.9	1.1	0.7	0.1
KIAA1580 protein	BAB13406	N.A.	N.A.	0.6	0.9	1.4	0.1
Mouse mRNA for P24 protein, complete cds.	D83206	NM_080723	N.A.	0.7	0.7	1.2	0.1
Mouse brain cDNA, clone MNCb-2875.	AB041807	N.A.	N.A.	0.8	0.7	1.0	0.1
Mouse apoptosis-related RNA binding protein (Napor-1) mRNA, complete cds.	AF090696	NM_006561	N.A.	0.9	0.7	0.7	0.1
Rat mRNA for protein tyrosine phosphatase epsilon M, partial cds.	D78613	NM_006504	N.A.	1.0	0.7	1.0	0.2
Rat mRNA for protein tyrosine phosphatase epsilon-like 2, partial.	Y07834	NM_006504	N.A.	1.0	0.7	1.0	0.1
Mouse cell death activator CIDE-A (Cide-a) mRNA, complete cds.	AF041376	NM_198289	N.A.	0.7	1.7	0.5	0.1
Mouse retinally abundant regulator of G-protein signaling mRGS-r (RGS-r) mRNA, complete cds.	U94828	NM_002928	N.A.	2.4	2.0	0.9	0.1
Rat clone C53 CDK5 activator-binding protein mRNA, complete cds.	AF177476	N.A.	Rn.3727	1.1	1.7	0.8	0.2
Rat calcium-independent alpha-latrotoxin receptor homolog 2 (CIRL-2) mRNA, complete cds.	AF063102	N.A.	Rn.12089	1.3	1.2	3.1	0.1
Rat calcium-independent alpha-latrotoxin receptor homolog 2 (CIRL-2) mRNA, complete cds.	AF063102	N.A.	Rn.12089	1.2	1.2	3.1	0.1
KIAA1132 protein	BAA86446	N.A.	N.A.	1.3	0.7	0.6	0.2
Rat purine-selective sodium/nucleoside cotransporter (rCNT2) mRNA, complete cds.	U66723	NM_004212	Rn.10140	0.3	0.8	0.8	0.2

Rat glycoprotein CD44 (CD44) mRNA, complete cds.	M61875	NM_000610	Rn.1120	0.1	0.6	0.4	0.1
Rat differentation-associated Na-dependent inorganic phosphate cotransporter (DNPI) mRNA, complete cds.	AF271235	N.A.	Rn.19372	0.1	1.0	0.4	0.1
Rat guanine nucleotide binding protein gamma subunit 11 mRNA, complete cds.	AF257110	NM_004126	Rn.892	0.3	0.7	0.2	0.1
Rat Ninj2 mRNA for ninjurin2, complete cds.	AB040815	NM_016533	Rn.35505	0.7	0.7	0.5	0.2
LOMP protein	AAD33924	N.A.	N.A.	0.5	0.2	0.1	0.1
Mouse carbonic anhydrase VII (Car7) mRNA, partial cds.	AF291660	N.A.	N.A.	0.5	0.4	0.1	0.1
CG2221 gene product	AAF46578	N.A.	N.A.	0.4	0.4	0.6	0.1
Mouse calcium- and diacylglycerol-regulated guanine nucleotide exchange factor I (CalDAG-GEFI) mRNA, complete cds.	U78171	NM_005825	N.A.	0.3	0.7	0.7	0.1
Rat mRNA for scraple responsive protein 1.	AJ132434	NM_007281	Rn.7724	0.3	0.2	0.4	0.1
aldo-keto reductase a	AAF42808	N.A.	N.A.	0.2	0.2	0.4	0.1
Mouse brain cDNA, clone MNCb-7013, similar to Mouse (Mus musculus) potassium channel Kv4.2 mRNA.	AB045326	NM_012281	N.A.	0.3	0.3	0.5	0.1
Rat mRNA for monocarboxylate transporter MCT2.	X97445	NM_004731	Rn.10524	0.3	0.3	0.4	0.1
Rat Apo D mRNA.	X55572	NM_001647	Rn.11339	0.4	0.5	0.2	0.1
Rat mRNA for transferrin, complete cds.	D38380	NM_001063	Rn.2514	0.3	0.4	0.2	0.1
Rat mRNA for Myelin-associated/Oligodendrocytic Basic Protein-81.	X89637	NM_182935	Rn.7619	0.2	0.2	0.1	0.0
Rat putative G-protein coupled receptor GPCR91 (Gpcr91) mRNA, complete cds.	AF090347	NM_057159	Rn.11200	0.2	0.2	0.1	0.0
Rat mRNA for carbonic anhydrase II.	X58294	NM_000067	Rn.26083	0.2	0.2	0.1	0.0
Mouse apoptosis-related RNA binding protein (Napor-3) mRNA, complete cds.	AF090697	NM_006561	N.A.	0.5	0.5	0.4	0.1
Rat mRNA for phosphodiesterase I.	D28560	NM_006209	Rn.20403	0.3	0.4	0.4	0.1
Mouse oligodendrocyte-specific protein mRNA, complete cds.	U19582	NM_005602	N.A.	0.4	0.4	0.3	0.1
Mouse mRNA for CA XIV, complete cds.	AB005450	N.A.	N.A.	0.4	0.5	0.3	0.1
Mouse mRNA for CA XIV, complete cds.	AB005450	N.A.	N.A.	0.4	0.5	0.2	0.1
Rattus norvegicus cDNA clone UI-R-DK0-cea-c-05-0-UI 3'.	BI294233	N.A.	Rn.43469	0.1	0.3	0.2	0.0
Mouse FKBP65RS-mRNA, complete cds.	AF279263	NM_007270	N.A.	0.4	1.0	0.2	0.1
Mouse ecotropic viral integration site 9 isoform C (Evi9) mRNA, complete cds.	AF169037	NM_022893	N.A.	0.3	0.6	0.1	0.1

Figure 14P

Figure 14Q

Rat Drosophila polarity gene (frizzled) homologue mRNA, complete cds.	L02529	N.A.	Rn.6575	0.3	0.8	0.1	0.1	
Rat Drosophila polarity gene (frizzled) homologue mRNA, complete cds.	L02529	N.A.	Rn.6575	0.4	1.1	0.2	0.1	
Mouse osf-2 mRNA for osteoblast specific factor 2, complete cds.	D13664	N.A.	N.A.	0.5	1.0	0.3	0.1	
bK984G1.1 (supported by FGENES)	CAB41538	N.A.	N.A.	1.2	0.8	0.1	0.1	
Mouse zinc finger protein (Mtsh1) mRNA, partial cds.	AF191309	NM_005786	N.A.	0.7	0.8	0.4	0.2	
hypothetical protein	CAB53247	N.A.	N.A.	0.9	1.0	0.2	0.1	
Mouse nuclear LIM-only 4 protein (Lmo4) mRNA, complete cds.	AF102817	NM_006769	N.A.	1.0	1.2	0.1	0.1	
Mouse mRNA encoding lysine-ketoglutarate reductase/saccharopine dehydrogenase.	AJ224761	NM_005763	N.A.	1.2	1.3	0.3	0.1	
Mouse ATP-binding cassette protein (Abca8) mRNA, partial cds.	AF213393	N.A.	N.A.	0.6	1.7	0.2	0.1	
RasGAP-related protein	AAB37765	N.A.	N.A.	0.5	0.6	0.1	0.1	
Mouse groucho-related protein (Grg2) mRNA, complete cds.	AF145958	NM_003260	N.A.	0.8	1.0	0.7	0.2	
Rat protein kinase C epsilon subspecies.	M18331	N.A.	N.A.	1.1	1.2	0.5	0.1	
Rat protein kinase C-binding protein NELL2 mRNA, complete cds.	U48245	NM_006159	Rn.11567	0.9	1.0	0.5	0.2	
Rat protein kinase C-binding protein NELL2 mRNA, complete cds.	U48245	NM_006159	Rn.11567	0.9	1.1	0.3	0.1	
Mouse mRNA for protein kinase C, complete cds.	D11091	NM_006257	N.A.	0.9	0.7	0.4	0.2	
Rat PKC theta mRNA, partial cds.	AB020614	N.A.	N.A.	0.9	0.7	0.4	0.2	
tuftelin	AAC04577	N.A.	N.A.	0.3	1.6	0.4	0.2	
Rat lipoprotein lipase mRNA, complete cds.	L03294	NM_000237	Rn.3834	0.1	2.0	0.1	0.0	
Rat cell adhesion regulator (CAR1) mRNA, complete cds.	U76714	NM_014437	Rn.15324	0.2	0.6	0.1	0.1	
Rat mRNA for leukoslalin.	Y00090	NM_003123	Rn.11144	0.3	0.9	0.2	0.1	
Rat CDK108 mRNA.	Y17328	NM_001888	Rn.24561	0.0	0.9	0.1	0.0	
Rat glia-derived nexin (GDN) mRNA, 5' end.	M17784	NM_006216	N.A.	1.1	0.4	0.5	0.2	
Rat osteoactivin mRNA, complete cds.	AF184983	NM_002510	Rn.13778	1.0	0.2	0.4	0.2	
Rat protein kinase C delta subspecies.	M18330	N.A.	N.A.	0.9	0.2	0.1	0.1	
A13 DA CLUSTER 2								

Figure 14R

DNA polymerase eta	AAD05272	N.A.	N.A.	0.4	0.3	1.2	0.1			
Rat Sam68-like protein SLM-2 (Slm-2) mRNA, complete cds.	AF152547	N.A.	Rn.52298	0.5	0.4	1.4	0.2			
HOMEOBOX PROTEIN SIX3	NM_023990	NM_005413		0.2	0.1	2.4	0.0			
Rat mRNA for Mac-MARCKS protein (F52 gene).	AJ301677	NM_023009	Rn.2486	0.3	0.7	1.4	0.1			
Rat phospholipase C-beta1b mRNA, complete sequence.	L14323	NM_182734	Rn.45523	0.4	0.6	1.5	0.1			
Rat phospholipase C-beta1b mRNA, complete sequence.	L14323	NM_182734	Rn.45523	0.4	0.6	1.7	0.1			
Rat mRNA for atypical PKC specific binding protein, complete cds.	AB005549	N.A.	Rn.31803	0.4	0.4	1.3	0.2			
Rat basic trancription element binding protein 2 (Bteb2) mRNA, complete cds.	AF182101	NM_001730	Rn.17478	0.5	0.5	1.9	0.2			
Rat PV-1 mRNA, complete cds.	AF154831	N.A.	Rn.53915	0.4	0.3	1.5	0.2			
Mouse homeobox protein Lim1 (Lim1) exons 1-4, complete sequence.	AF039705	N.A.	N.A.	0.3	0.2	2.1	0.2			
Mouse prospero-related homeobox 1 (Prox1) mRNA, complete cds.	AF061576	NM_002763	N.A.	0.5	0.4	1.0	0.1			
KIAA1613 protein similar to cationic amino acid transporter	BAB13439	N.A.	N.A.	0.1	0.3	2.6	0.7			
Mouse mRNA for forkhead protein FKHR (Fkhr gene).	AJ252157	N.A.	N.A.	0.1	0.2	1.1	0.4			
cDNA clone similar to voltage-dependent calcium channel, alpha 1I subunit	BE095865	N.A.	Rn.21852	0.1	0.2	1.3	0.3			
tetraspan TM4SF; Tspan-1.	AAC69714	N.A.	N.A.	0.2	0.3	1.1	0.4			
Mouse partial mRNA for stretch response protein 553 (sr553 gene).	AJ250188	N.A.	N.A.	0.2	0.2	1.4	0.2			
Rat mRNA for glutamic acid decarboxylase.	X57573	NM_000817	Rn.10370	0.1	0.0	1.3	0.1			
Mouse zinc transporter ZnT-3 (ZnT-3) mRNA, complete cds.	U76007	NM_003459	N.A.	0.0	0.0	1.7	0.0			
Mouse gene for Arx homeodomain protein, exon 5 and complete cds.	AB026674	N.A.	N.A.	0.0	0.1	5.9	0.0			
Mouse mRNA, one isoform of PTP-RL9.	D83204	NM_002843	N.A.	0.1	0.1	0.4	0.1			
Rat mRNA encoding rat pre-prosomatostatin (isolated from a medullary thyroid carcinoma).	V01271	NM_001048	Rn.34418	0.1	0.1	0.4	0.1			
Rat insulin-like growth factor binding protein 5 mRNA, 3' UTR.	AF139830	In	Rn.1593	0.2	0.6	1.7	0.2			

Figure 14S

Rat neuropilin mRNA, complete cds.	AF016296	N.A.	Rn.10815	0.1	0.3	0.8	0.1
Mouse COUP-TF1.	X74134	NM_005654	N.A.	0.1	0.1	0.3	0.1
Mouse zic mRNA for Zic protein, complete cds.	D32167	NM_003412	N.A.	0.0	0.0	0.1	0.0
Mouse ets-related protein 81 (ER81) mRNA, complete cds.	L10426	NM_004956	N.A.	0.1	0.1	0.5	0.2
Rat ryanodine receptor type 2 mRNA, partial cds.	AF112257	NM_001035	N.A.	0.1	0.1	0.4	0.2
Rat nerve growth factor-induced (NGFI-A) gene, complete cds.	M18416	NM_001964	Rn.9096	0.0	0.1	0.1	0.0
Cricetinae gen. sp. insulin receptor tyrosine kinase 53 kDa substrate mRNA, complete cds.	U41899	N.A.	N.A.	0.1	0.1	0.4	0.4
Mouse hormonally upregulated neu tumor-associated kinase (Hunk) mRNA, complete cds.	AF167987	NM_014586	N.A.	0.1	0.1	0.6	0.3
Mouse mSox5L mRNA, complete cds.	AB006330	NM_152989	N.A.	0.1	0.2	0.7	0.3
Mouse mSox5L mRNA, complete cds.	AB006330	NM_152989	N.A.	0.1	0.2	0.8	0.3
Rat IgE binding protein mRNA, complete cds.	J02962	NM_194327	Rn.764	0.3	0.2	2.2	0.5
Mouse mRNA for K-glypican.	X83577	NM_001448	N.A.	0.1	2.3	0.3	0.3
Mouse mRNA for entactin.	X14194	N.A.	N.A.	0.1	2.1	0.3	0.6
Rattus norvegicus chloride intracellular channel 5 (Clic5), mRNA. NM_053603	NM_053603	NM_016929	Rn.1838	0.2	1.3	0.6	0.6
Rattus norvegicus cDNA clone UI-R-DR0-cjc-g-03-0-UI 3'.	BI303966	N.A.	Rn.49567	0.1	0.7	0.6	0.5
Rat synaptotagmin interacting protein STIP2 mRNA, complete cds.= neuronal calcium binding protein 2	AF193757	N.A.	Rn.15796	0.2	1.3	1.3	0.3
Rat testis-specific histone H1t and histone H4t, complete cds.	M28409	N.A.	Rn.48806	1.0	0.3	0.2	0.6
Rat Sprague-Dawley N-methyl-D-aspartate receptor NMDAR2C subunit mRNA, complete cds.	U08259	NM_000835	Rn.9709	0.7	0.1	0.2	0.3
Mouse mRNA for uracil-DNA glycosylase.	X99018	NM_003362	N.A.	1.4	0.4	0.2	0.8
Rat mRNA for HB2, complete cds.	AB008538	NM_001627	Rn.5789	0.4	0.9	0.2	0.4
Mouse c-kit mRNA for truncated tyrosine-kinase.	X65997	NM_000222	N.A.	0.5	0.4	0.1	0.4
Mouse c-kit mRNA for truncated tyrosine-kinase.	X65997	NM_000222	N.A.	0.4	0.4	0.1	0.4
Mouse mRNA, up-regulated by FUS-ERG, 3' region, cDNA fragment: C14G220.	AB028209	NM_014572	N.A.	0.5	0.5	0.1	0.4
Mouse renal munc13 mRNA, complete cds.	AF115848	AB028955	N.A.	0.7	0.8	0.2	0.4
Rat KCNMB4 mRNA for calcium activated potassium channel beta 4 subunit, complete cds.	AB050637	NM_014505	Rn.64491	0.7	0.7	0.1	0.6
calmodulin	AAD56955	N.A.	N.A.	0.1	0.2	0.1	0.2

Figure 14T

Rat RIM binding protein 2 (Rbp2) mRNA, partial cds.	AF199336	N.A.	Rn.54192	1.0	0.6	0.2	1.4
239FB gene product	AAC50564	N.A.	N.A.	1.3	1.3	0.2	1.1

Figure 15A

Gene name	GB Acc.	Human Orthologs	Unigene	RATIO SN	RATIO VTA	RATIO A13	RATIO LC
Wnt2: wingless-type MMTV integration site 2	AK045120	NM_003391	N.A.	2.5	9.3	0.1	1.6
unnamed protein product	BAB14897	N.A.	N.A.	3.3	7.8	3.8	2.5
Mouse lysyl hydroxylase isoform 2 mRNA, complete cds.	AF080572	NM_182943	N.A.	4.4	19.1	0.8	1.9
Mouse NMRI fibroglycan (syndecan-2) gene, complete cds.	U00674	NM_002998	N.A.	3.1	8.7	1.9	1.5
Rat core protein (HSPG) mRNA, complete cds. =syndecan 2	M81687	NM_002998	Rn.11127	3.4	8.8	2.0	1.7
Rat serine protease RNK-Met-1 mRNA, complete cds . solute carrier family 39 (zinc transporter), member 4 (ZIP-4)	L05175	NM_005317	Rn.9838	1.8	4.7	0.8	0.6
bA524D16A.1 (sushi-repeat-containing protein)	BQ196656	NM_017767	Rn.7960	103.6	265.6	1.6	1.4
Rat kidney extracellular calcium-sensing receptor mRNA, complete cds.	CAC16060	N.A.	N.A.	12.5	37.1	1.2	1.3
Rattus norvegicus cDNA clone UI-R-BT1-akv-g-05-0-UI 3'.	U10354	NM_000388	Rn.10019	0.9	16.8	0.9	0.2
Mouse C1q-related factor mRNA, complete cds.	AW531194	N.A.	Rn.46621	1.1	3.5	7.3	6.0
Rat vanilloid receptor splice variant mRNA, complete cds.	AF095155	N.A.	N.A.	1.2	3.3	4.8	7.5
Rat mRNA for Castration Induced Prostatic Apoptosis Related protein-1 (CIPAR-1).	AF158248	NM_080706	Rn.3073	1.0	3.8	1.1	1.6
hypothetical protein	AJ010750	N.A.	Rn.21667	1.0	5.0	7.4	1.2
Mouse myristoylated alanine-rich C-kinase substrate (MARCKS) mRNA, complete cds.	CAB94883	N.A.	N.A.	0.7	4.1	0.4	0.4
Rattus norvegicus cDNA clone UI-R-DK0-ceq-g-09-0-UI 3' similar to hypothetical protein DKFZp564B1162 with RHOGAP domain	M60474	NM_002356	N.A.	0.9	2.2	2.7	1.1
Mouse bone morphogenetic protein 2 (BMP-2) gene, complete cds.	BI295380	N.A.	Rn.13264	0.9	2.0	1.5	4.1
Rat bone morphogenetic protein 2 related mRNA sequence.	L25602	N.A.	N.A.	1.2	3.8	0.2	1.5
Rat follistatin gene, exon 6, clones pROF(301-305).	L20678	NM_001200	Rn.12687	1.0	2.6	0.3	1.4
axonemal dyneln heavy chain	M31591	N.A.	N.A.	0.6	10.5	1.9	0.4
	CAB94756	N.A.	N.A.	1.0	2.6	1.0	4.0

Figure 15B

Mouse Ephrin B3	AK048305	NM_001406	N.A.	0.9	3.1	3.6	1.0
Rat gene for hepatocarcinogenesis-related transcription factor (HTF), complete cds.	AB030238	N.A.	Rn.13157	1.1	2.2	1.1	1.2
unnamed protein product	BAA91922	N.A.	N.A.	1.3	3.5	0.2	0.4
Rat phospholipase C gamma-2 , complete cds.	J05155	NM_002661	Rn.9751	1.2	8.5	1.0	0.4
Rat DRG Library Rattus norvegicus cDNA clone DRNBLF02 5'.	BG671101	N.A.	Rn.3291	1.2	5.3	11.0	1.8
Myopodin protein	CAB51856	N.A.	N.A.	1.2	3.2	0.4	0.2
Mouse mRNA for distal intestinal serine protease (DISP gene).	AJ243866	N.A.	N.A.	1.4	4.4	0.8	2.0
Rat huntingtin associated protein (rHAP1-B) mRNA, complete cds, alternatively spliced form.	U38370	NM_003949	Rn.37430	1.3	3.7	8.2	5.5
Rattus norvegicus cDNA clone UI-R-C0-hi-d-02-0-UI 3' similar to Necdin-like protein 1 and melanoma antigen, family L, 2 (Magel2), involved in Prader-Willi syndrome	AA996569	N.A.	N.A.	1.6	5.3	10.8	4.2
Rat mRNA for novel gene expressed in circadian manner, clone SCN8.	X95850	NM_001627	Rn.7363	1.3	3.0	0.5	1.2
Rat alpha-1B adrenergic receptor mRNA, complete cds.	M60655	NM_000679		1.5	3.8	0.8	0.2
Mouse mRNA for Rho guanine nucleotide-exchange factor, splice variant NET1A.	AJ010045	NM_005863	N.A.	1.2	2.6	0.3	0.3
Mouse hepatocyte growth factor activator inhibitor type 2 splice variant 2 (Hai2) mRNA, complete cds.	AF099020	NM_021102	N.A.	1.1	2.6	2.9	1.1
Rat pancreatic ribonuclease mRNA.	J00771	N.A.	N.A.	1.0	5.0	0.9	0.7
palate, lung, and nasal epithelium expressed transcript (Plunc)	U69172	NM_130852	N.A.	0.6	9.1	6.7	5.3
Mouse DNA for Gpx2 pseudogene.	X91864	N.A.	N.A.	51.0	13.3	1.3	2.8
Rattus norvegicus cDNA clone UI-R-C0-gx-g-08-0-UI 3'.	AA964745	N.A.	Rn.11902	6.6	2.5	0.9	0.8
Rat mRNA for sensory neuron synuclein.	X86789	NM_003087	Rn.10421	28.1	4.3	1.8	29.5
G-protein-coupled receptor GPRC5C	NM_181444	Mm.23575	7.8	2.0	2.0	1.9	0.2
Rat mRNA for fibromodulin.	X82152	NM_002023	Rn.8778	0.0	0.3	0.1	0.0
Rattus norvegicus cDNA clone UI-R-DK0-cea-c-05-0-UI 3'.	BI294233	N.A.	Rn.43469	0.1	0.3	0.2	0.3

Figure 15C

cDNA clone similar to voltage-dependent calcium channel, alpha 1I subunit	BE095865	N.A.	Rn.21852	0.1	0.2	1.3	0.7	
KIAA1613 protein similar to cationic amino acid transporter	BAB13439	N.A.	N.A.	0.1	0.3	2.6	0.1	
hypothetical Leucine-rich repeat, typical subtype containing protein	BE096686	N.A.	Rn.48962	0.1	0.4	0.1	0.9	
Rat neuronal olfactomedin-related ER localized protein (D2Sut1e) mRNA, complete cds.	U03417	NM_014279	Rn.11005	0.1	0.4	0.7	0.1	
Mouse (E25) mRNA, complete cds.	L38971	NM_004867	N.A.	0.2	0.5	0.2	0.1	
Mouse integral membrane protein 2A (Itm2a) gene, complete cds.	AF074020	N.A.	N.A.	0.2	0.5	0.2	0.1	
Rattus norvegicus G-substrate phosphatase inhibitor mRNA, complete cds.	AF294688	N.A.	Rn.24323	0.1	0.3	0.1	0.0	
Rat nerve growth factor-induced (NGFI-A) gene, complete cds.	M18416	NM_001964	Rn.9096	0.0	0.1	0.1	1.2	
Rattus norvegicus cDNA clone UI-R-CV0-brm-d-06-0-UI 3'.	BG372104	N.A.	Rn.22267	0.1	0.3	1.0	0.1	
Rat neuropilin mRNA, complete cds.	AF016296	N.A.	Rn.10815	0.1	0.3	0.8	0.9	
Mouse zinc metalloendopeptidase ADAMTS8 mRNA, complete cds.	AF175282	NM_007037	N.A.	0.3	0.8	0.4	0.2	
Rat E11 antigen epitope (OTS-8) mRNA, complete cds.	U32115	N.A.	Rn.794	0.2	0.6	0.3	0.2	
Rat mRNA for RB109 (brain specific protein), complete cds.	D26154	N.A.	N.A.	0.4	0.9	0.7	0.1	
Mouse calcium- and diacylglycerol-regulated guanine nucleotide exchange factor I (CalDAG-GEFI) mRNA, complete cds.	U78171	NM_005825	N.A.	0.3	0.7	0.7	0.2	
Rat mRNA for collagen alpha 2 type V, partial cds.	AJ224880	NM_000393	Rn.2875	0.1	0.6	0.2	0.2	
Rat insulin-like growth factor binding protein 5 mRNA, 3' UTR.	AF139830	multiple cluste	Rn.1593	0.2	0.6	1.7	0.4	
Mouse brain cDNA, clone MNCb-2717.	AB041591	NM_153357	N.A.	0.3	0.6	0.9	0.2	
Rattus norvegicus cDNA clone UI-R-CN1-cjm-g-13-0-UI 3'.	BM390981	N.A.	Rn.23137	0.2	0.7	0.2	0.5	
Mouse cAMP inducible 1 protein (Ci1) mRNA, complete cds.	AF121080	NM_016582	N.A.	0.3	0.7	0.4	0.5	

Figure 15D

Rattus norvegicus cDNA clone UI-R-DR0-cjc-g-03-0-UI 3'.	BI303966	N.A.	Rn.49567	0.1	0.7	0.6	0.0	
Rat CDK108 mRNA.	Y17328	NM_001888	Rn.24561	0.0	0.9	0.1	0.3	
extensin-like protein	CAA84230	N.A.	N.A.	0.3	0.9	0.4	0.7	
Mouse sprouty 1 (Spry1) mRNA, complete cds.	AF176903	NM_005841	N.A.	0.3	0.9	0.7	0.2	
Rat purine-selective sodium/nucleoside cotransporter (rCNT2) mRNA, complete cds.	U66723	NM_004212	Rn.10140	0.3	0.8	0.8	0.1	
Rat differentation-associated Na-dependent inorganic phosphate cotransporter (DNPI) mRNA, complete cds.	AF271235	N.A.	Rn.19372	0.1	1.0	0.4	0.4	
Mus musculus, claudin 1, clone IMAGE:3493550, mRNA	BC008536	NM_021101	N.A.	0.5	2.0	0.3	0.6	
Rattus norvegicus cDNA clone UI-R-C0-jq-d-07-0-UI 3'.	AI045808	N.A.	Rn.44028	0.3	2.7	1.4	1.4	
Rat pituitary adenylate cyclase activating polypeptide precursor protein mRNA, complete cds.	M63006	NM_001117	Rn.37400	0.2	4.0	0.3	0.9	
Rattus norvegicus cDNA clone UI-R-C0-gs-c-02-0-UI 3'.	AA963971	N.A.	Rn.7936	0.5	2.3	0.5	2.2	
Mouse RNA guanylyltransferase (Mce1) mRNA, complete cds.	AF034568	NM_003800	N.A.	0.6	2.2	1.6	10.8	
Rat mRNA for Rab3B protein.	Y14019	NM_002867	Rn.3788	0.7	2.4	2.3	0.7	
Mouse chordin mRNA, complete cds.	AF096276	NM_177978	N.A.	0.6	1.8	1.0	0.4	
Rattus norvegicus similar to HTPAP protein, mRNA, contains acid phosphatase domain	XM_219376	N.A.	N.A.	0.4	3.8	1.1	1.2	
Rat mRNA for Mx3 protein.	X52713	NM_002463	Rn.10374	0.7	2.0	0.5	4.6	
Rattus norvegicus cDNA clone RGIDW96	AW916943	N.A.	Rn.91509	0.7	1.8	2.2	0.0	
Rat lipoprotein lipase mRNA, complete cds.	L03294	NM_000237	Rn.3834	0.1	2.0	0.1	5.0	
similar to AD037 protein containing RalGDS/AF-6 domain	BI293840	N.A.	N.A.	0.5	1.4	1.0	0.8	
immunoglobulin superfamily, member 4, IGSF4	AF434663	NM_014333	Mm.248549	0.4	1.7	0.7	0.9	
immunoglobulin superfamily, member 4, IGSF4	AF434663	NM_014333	Mm.248549	0.5	1.8	0.7	1.2	
Rat GlcAT-S mRNA for UDP-glucuronyltransferase-S, complete cds.	AB010441	N.A.	Rn.42869	0.6	2.6	2.5	0.1	
Mouse ATP-binding cassette protein (Abca8) mRNA, partial cds.	AF213393	N.A.	N.A.	0.6	1.7	0.2	1.3	

Figure 15E

Mouse mRNA for adhesion protein RA175N, complete cds.	AB021967	NM_014333	N.A.	0.5	2.0	0.8	1.1	
Mouse manic fringe precursor mRNA, complete cds. similar to AD037 protein containing RalGDS/AF-6 domain	U94349	NM_002405	N.A.	0.5	2.1	1.0	5.8	
Mouse neurogenic extracellular slit protein (Slit2) mRNA, partial cds.	XM_232305	N.A.	N.A.	0.6	1.5	1.0	2.0	
Rat fibrillin-1 mRNA, complete cds.	AF074960	NM_004787	N.A.	0.6	1.7	1.4	2.6	
Rat AMP deaminase (ampd2) gene, partial cds.	AF135059	NM_000138	Rn.12759	0.6	1.8	1.1	2.3	
Rat PND gene encoding atrial natriuretic factor, complete cds.	M38126	N.A.	N.A.	0.7	1.4	2.0	2.9	
Mouse mRNA for Doc2beta, complete cds.	K02062	N.A.	Rn.2004	0.5	1.8	14.1	3.1	
Mouse mRNA for K-glypican.	D85037	NM_003585	N.A.	0.4	1.6	2.0	0.3	
Rat Drosophila polarity gene (frizzled) homologue mRNA, complete cds.	X83577	NM_001448	N.A.	0.1	2.3	0.3	0.1	
Rat SOD gene, complete cds.	L02529	N.A.	Rn.6575	0.4	1.1	0.2	0.4	
Rat synaptotagmin interacting protein STIP2 mRNA, complete cds.	Z24721	NM_003102	Rn.10358	0.5	1.2	0.4	0.3	
POZ/zinc finger transcription factor ODA-8	AF193757	N.A.	Rn.15796	0.2	1.3	1.3	1.3	
tuftelin	AF194030	N.A.	Mm.234644	0.5	1.1	1.3	0.2	
Mouse mRNA for entactin.	AAC04577	N.A.	N.A.	0.3	1.6	0.4	0.6	
Rattus norvegicus chloride intracellular channel 5 (Clic5), mRNA. NM_053603	X14194	N.A.	N.A.	0.1	2.1	0.3	0.6	
Rat heat shock protein 22 mRNA, complete cds.	NM_053603	NM_016929	Rn.1838	0.2	1.3	0.6	2.3	
Sox6 (SRY-box containing gene 6)	AF314540	N.A.	Rn.12650	1.8	0.6	3.2	0.2	
DRNBTE01 Rat DRG Library Rattus norvegicus cDNA clone DRNBTE01 5'.	AK044981	NM_033326	Mm.4656	2.3	0.6	0.4	0.3	
Mouse erythroid differentiation related factor mRNA, complete cds.	BG673363	N.A.	N.A.	3.4	0.9	0.3	1.1	
Rat Sprague-Dawley N-methyl-D-aspartate receptor NMDAR2C subunit mRNA, complete cds.	AF060220	NM_016633	N.A.	3.2	0.6	0.8	0.3	
Rat mRNA for receptor activity modifying protein 1, complete cds.	U08259	NM_000835	Rn.9709	0.7	0.1	0.2	0.4	
Rat regulator of G-protein signaling protein 2 mRNA, complete cds.	AB642887	NM_005855	Rn.12265	1.0	0.4	0.4	0.6	
	AF279918	NM_002923	Rn.1892	1.0	0.4	0.9	0.6	

Figure 15F

Rat testis-specific histone H1t and histone H4t, complete cds.	M28409	N.A.	Rn.48806	1.0	0.3	0.2	0.1
Rat protein kinase C delta subspecies.	M18330	N.A.	N.A.	0.9	0.2	0.1	0.3
Rat protein kinase C delta subspecies.	M18330	N.A.	N.A.	1.1	0.4	0.4	0.2
Rat osteoactivin mRNA, complete cds.	AF184983	NM_002510	Rn.13778	1.0	0.2	0.4	0.1
LOMP protein	AAD33924	N.A.	N.A.	0.5	0.2	0.1	0.1

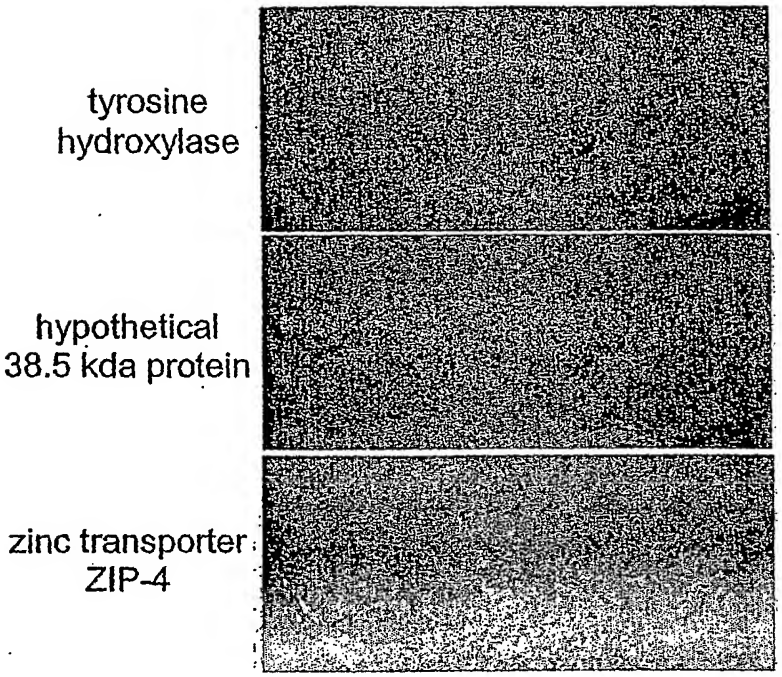

tyrosine hydroxylase
hypothetical 38.5 kda protein
zinc transporter ZIP-4

Figure 16